

MORPHOLOGICAL AND PHENOLOGICAL RESPONSES OF BUTTERFLIES TO
SEASONAL TEMPERATURE INCREASE IN ALASKA

By Kathryn Margaret Daly, B. S.

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of
Master of Science
in
Biological Sciences

University of Alaska Fairbanks

December 2018

Dr. Greg A. Breed, Committee Co-chair

Dr. Derek S. Sikes, Committee Co-chair

Dr. Daniel H. Mann, Committee Member

Dr. Diane Wagner, Chair

Department of Biology and Wildlife

Dr. Leah Berman, Dean

College of Natural Sciences and Mathematics

Dr. Michael Castellini

Dean of the Graduate School

Abstract

Climate is changing rapidly at high latitudes, and the responses of insects provide early indications of the impacts these changes have on biota. Butterflies (Lepidoptera: Papilionoidea) are among the best-known Subarctic and Arctic insects, and research in Greenland has revealed significant declines in butterfly body sizes along with advances in the timing of their first flights in spring. These changes are ecologically significant because smaller body sizes can lead to reduced fecundity in butterflies, and earlier adult emergence can have detrimental effects across trophic levels because Lepidoptera are an important food resource for birds and mammals. The primary goal of this thesis is to expand the geographical scope of previous studies of butterfly responses to high-latitude warming by testing whether Alaskan butterflies have exhibited morphological (Chapter 1) and/or phenological (Chapter 2) changes in response to rising temperatures. The morphological parameter studied here is forewing length, and the phenological parameter the timing of the first-observed flight of the year. Results show that the wings of two out of three butterfly species studied from Alaska's North Slope and Seward Peninsula decreased as seasonal (spring and summer) temperatures rose between 1971 and 1995. For every 1° C increase in average seasonal temperatures, wingspans decreased by up to 1.4 millimeters in Alaska. This compares to decreases of up to 0.65 millimeters observed in Greenland. One Alaskan species, *Colias hecla* Lefebvre 1836, did not show significant change in its wing lengths, although it did exhibit significant decreases in Greenland. Differences in life-history traits among species appear to result in divergent responses in Alaskan butterflies, with *Boloria freija* (Thunberg, 1791), which overwinters as late-instar larvae, showing the greatest decrease in wing length compared to *Boloria chariclea* (Edwards, 1883) which overwinters as early-instar larvae. From the start of the collection record in 1966 onward, collection and

observational records from Interior Alaska reveal an average phenological advancement of 1 to 5 days/decade in 13 spring-emerging butterfly species. The morphological and phenological changes found in some species of Alaskan butterflies correlate with recent climate change, though the effects differed among species. The eco-physiological responses to climate change observed here for butterflies are likely to be shared by other insect species living at high latitudes.

Table of Contents	Page
Abstract	i
List of Figures.....	vii
List of Tables.....	ix
List of Appendices	x
Acknowledgements.....	xi
General Introduction	1
Chapter 1 : Wing Length In Alaskan Butterflies Decreases During Warmer Summers Across A Broad Geographic Range	7
Abstract.....	7
Introduction.....	8
Methods	11
Specimen & Species Data	11
Regional Data	12
Measurement Data	14
Temperature Data	14
Standardization Of Temperature Data	15
Statistical Analysis.....	23
Results	23
Boloria freija.....	26

<i>Boloria chariclea</i>	28
<i>Colias hecla</i>	30
Discussion	32
Literature Cited	35
Chapter 2 : Phenological Advancement Of Early-Season Interior, Alaska Butterfly Species From 1966-2016.....	39
Abstract.....	39
Introduction.....	40
Methods	43
Study Species and Data Sources.....	43
Specimen Identification	44
Defining Phenological Shift.....	46
Temperature Data	46
Statistical Analysis.....	47
Results	48
Discussion.....	55
Literature Cited	59
General Discussion	66
Appendix	69
Literature Cited	81

List of Figures

Chapter 1	Page
Figure 1: Localities where butterfly specimens were collected (blue) and locations of weather stations (red), along with the region (SP = Seward Peninsula, W. NS= western North Slope, E. NS= eastern North Slope) which specimens were grouped in as a random effect in analysis.....	13
Figure 2. Alaskan weather stations in operation on the North Slope between 1970 and 1995, separated by the regions depicted in Fig. 1 showing continuity of coverage between coastal eastern stations (blue), inland eastern stations (orange), and coastal western stations (grey), and inland western stations (yellow)	16
Figure 3. Mean daily temperature (°C) for eastern-North-Slope-inland (red) and coastal-weather stations (black) between 1970 – 1995, located between 155°W and 142°W longitude	18
Figure 4. Mean daily temperature (°C) for Alaskan western North Slope inland (red) and coastal weather stations (black) between 1970 – 1995, located between 167°W and 155°W longitude..	20
Figure 5. Seasonal collection records combined for the Seward Peninsula and the eastern and western North Slope for <i>Colias hecla</i> (top) <i>Boloria chariclea</i> (middle) and <i>Boloria freija</i> (bottom) by Day of Year (DOY). The first flights of both <i>Colias hecla</i> and <i>Boloria chariclea</i> began around DOY 180 (29 June), whereas the majority of <i>Boloria freija</i> were collected after DOY 160 (9 June) from 1971-1995.....	22
Figure 6. <i>Boloria freija</i> forewing length measurements compared with growing degree days (GDD) during the previous summer (left column) and during the spring of collection year (right column) for females (pink) and males (blue) on the Seward Peninsula (top), western North Slope (center), and eastern North Slope (bottom).....	27
Figure 7. <i>Boloria chariclea</i> forewing length measurements compared with growing degree days (GDD) during the previous summer (left column) and during the spring of collection year (right column) for females (pink) and males (blue) on the Seward Peninsula (top), western North Slope (center), and eastern North Slope (bottom).....	29
Figure 8. <i>Colias hecla</i> forewing length measurements compared with growing degree days (GDD) during the previous summer (left column) and during the spring of collection year (right column) for females (pink) and males (blue) on the Seward Peninsula (top), western North Slope (center), and eastern North Slope (bottom).....	31

List of Figures (cont'd)

Chapter 2	Page
<p>Figure 1. Location of the weather station at Fairbanks International Airport (red star) and the butterfly records (blue dots) for the Fairbanks area derived from the Global Biodiversity Information Facility (GBIF) for <i>Papilio canadensis</i>, <i>Papilio machaon</i>, <i>Erynnis persius</i>, <i>Carterocephalus palaemon</i>, <i>Glaucopsyche lygdamus</i>, <i>Celastrina lucia</i>, <i>Callophrys polios</i>, <i>Callophrys augustinus</i>, <i>Colias canadensis</i>, <i>Pontia occidentalis</i>, <i>Boloria frigga</i>, <i>Boloria freija</i>, <i>Erebia discoidalis</i>, <i>Erebia epipsodea</i>, <i>Erebia mancinus</i> documented from 1966 onward. Study area within Alaska (Panel A) near Fairbanks (Panel B).....</p>	45
<p>Figure 2. Annual accumulated growing degree days (GDD) calculated from a base of 0 °C for Fairbanks, Alaska from 1930 to 2016 (left). Since 1930 GDD have increased by 42.8 GDD \pm 6.2 SE per decade (adjusted R-squared = 0.3473, F = 46.76, p < 0.001). Between 1966 and 2016, cumulative growing degree days °C as of 30 June of each year increased 18.7 \pm 0.81SE per decade (Adjusted R-squared = 0.07878, p= 0.0259)</p>	49
<p>Figure 3. Spring emergence trends for species in Fairbanks, Alaska: <i>Papilio canadensis</i> (left column) records (top left) depicting all records which caused significant influence on the linear regression prediction; leverage values ranging from 3% are shown in red. <i>P. canadensis</i> records from 1966-2005 (bottom left) were subsequently analyzed. <i>Papilio machaon</i> records (top right) were analyzed from 1966-2005 and <i>Carterocephalus palaemon</i> (bottom right) were analyzed from 1966-1990</p>	52

List of Tables

Chapter 1	Page
Table 1. Butterfly specimen sample size, including total measured individuals (n=2501) by species (rows) and region of Alaska (columns)	12
Table 2: Comparison of linear mixed effect models testing butterfly wing length as a function of sex, species, and spring and/or previous summer GDD values with Akaike Information Criterion (AIC) score depicted as change from the best fitting (lowest scoring) model. GDD data were not included in null model, but all models included a random effect of region.....	24
Chapter 2	Page
Table 1. Species records for Fairbanks, specimen records obtained from collections including (1) Kenelm W. Philip Lepidoptera Collection (KWP), (2) University of Alaska Museum (UAM), (3) Utah Museum of Natural History (UMNH), (4) Oklahoma Museum of Natural History (OMNH) and the (5) McGuire Center for Lepidoptera and Biodiversity (MGCL). Observational records obtained from (6) iNaturalist / eButterfly. Significant advancement trends (days per year) : p value <0.05 (*), p <0.01 (**), p<0.001 (***).	50

List of Appendices

Appendix: Chapter 2	Page
Figure A1. <i>Erynnis persius</i> records from 1966-1990 for Fairbanks, Alaska revealed a non-significant advancement of 3.8 ± 1.4 SE days per decade ($R^2 = 0.05146$, $F = 7.052$, $p = 0.008906$)	69
Figure A2. <i>Colias canadensis</i> in Fairbanks, Alaska from 1970 – 2005 exhibited a strong advancement of 4.7 ± 0.6 SE days per decade ($R^2 = 0.1002$, $F = 56.82$, $p < 0.001$)	70
Figure A3. <i>Pontia occidentalis</i> in Fairbanks, Alaska from 1966-2016 showed a non-significant delay in flight period, flying 2.2 ± 1.4 SE days later per decade ($R^2 = 0.05944$, $F = 2.275$, $p = 0.1402$) over 51 years.....	71
Figure A4. <i>Celastrina lucia</i> in Fairbanks, Alaska from 1966-2016 had a non-significant change in flight period over 51 years of 0.7 ± 0.5 SE days later per decade ($R^2 = 0.006735$, $F = 1.553$, $p = 0.214$)	72
Figure A5. <i>Glaucopsyche lygdamus</i> in Fairbanks, Alaska from 1966-2016 advanced 1.4 ± 0.4 SE days per decade ($R^2 = 0.03035$, $F = 10.77$, $p = 0.001138$).....	73
Figure A6. <i>Callophrys polios</i> in Fairbanks, Alaska from 1966 – 2005 advanced 1.0 ± 0.4 SE days per decade ($R^2 = 0.01369$, $F = 5.4$, $p = 0.02065$).....	74
Figure A7. <i>Callophrys augustinus</i> in Fairbanks, Alaska from 1966-2005 had an early flight trend of 2.0 ± 0.4 SE days per decade ($R^2 = 0.06262$, $F = 20.17$, $p < 0.001$)	75
Figure A8. <i>Boloria frigga</i> in Fairbanks, Alaska from 1966-2005 advanced 1.3 ± 0.3 SE days per decade ($R^2 = 0.06089$, $F = 18.28$, $p < 0.001$).....	76
Figure A9. <i>Boloria freija</i> in Fairbanks, Alaska from 1966 – 2005 had an advancement of 1.8 ± 0.4 SE days per decade ($R^2 = 0.0485$, $F = 19.83$, $p < 0.001$)	77
Figure A10. <i>Erebia discoalalis</i> in Fairbanks, Alaska from 1966-2016 advanced 2.1 ± 0.3 SE days per decade ($R^2 = 0.1356$, $F = 37.95$, $p < 0.001$).....	78
Figure A11. <i>Erebia epipsodea</i> in Fairbanks, Alaska from 1966-2016 advanced 1.5 ± 0.2 SE days per decade ($R^2 = 0.2848$, $F = 40.62$, $p < 0.001$).....	79
Figure A12. <i>Erebia mancinus</i> in Fairbanks, Alaska from 1966-2016 advanced 2.5 ± 0.3 SE days per decade ($R^2 = 0.2218$, $F = 51.31$, $p < 0.001$) from 1966-2017.....	80

Acknowledgements

This research was made possible by the Lepidoptera collection of my late mentor, Dr. Kenelm Winslow Philip (6 November 1931 – 13 March 2014), founder of the Alaska Lepidoptera Survey. His kindness and good humor inspired me to pursue butterflies in all senses of the word, and this thesis is dedicated to his memory. It has been my fortune to have the help of many talented researchers both at the University of Alaska Fairbanks and beyond. I gratefully acknowledge the guidance and support of my core committee, including co-advisors Derek Sikes and Greg Breed, along with Dan Mann. I thank Roman Dial for introducing me to R as a tool for investigation of butterfly phenology. I am grateful for insight and encouragement from Cliff Ferris and Andy Warren throughout this process. This research was made possible through the help of fifteen undergraduate biology students at the University of Alaska Fairbanks who measured specimens during the Spring 2016 semester. I thank Jayce Williamson for her meticulous laboratory assistance in processing specimens with me for the morphology component of this research, which was funded through the 2016 Kenelm W. Philip Entomology Research Award. Additionally, I thank Jayce Williamson, Renee Nowicki, and Karissa Amador for their patience and diligence in digitizing the Kenelm W. Philip Collection.

General Introduction

Global climate change has caused warming temperatures in Arctic tundra and boreal forest ecosystems. Mean annual air temperatures have risen significantly over the last century at high latitudes in the northern hemisphere ($>64^{\circ}\text{N}$), with 2016 being the hottest year since temperature records began in AD 1900 (Richter-Menge *et al.* 2016). Based on satellite imagery collected between 1967 and 2012, Comiso and Hall (2014) detected declining spring snow cover (-2.12% per decade) and estimated that since 1981 the Arctic has warmed at a rate of $\sim 0.60 \pm 0.07^{\circ}\text{C} / \text{decade}$, which is three times the global average of $0.17^{\circ}\text{C} / \text{decade}$. Warming trends are unequal between seasons, with the greatest warming occurring in spring and winter (Rigor *et al.* 2000, Przybylak 2007).

Rising temperatures can have strong impacts on flora and fauna. Warming in Alaska has been accompanied by an overall trend of Arctic greening, an increase in plant chlorophyll which can be detected through satellite imagery (Ju & Masek, 2016). In the Alaskan tundra, vegetation composition has changed in structure due to an expansion of shrubs over the last half century (Sturm *et al.* 2001). While the Arctic tundra in Alaska has greened, a decline in productivity, or browning, has occurred within the boreal forests of Interior Alaska (Goetz *et al.* 2005). The extent of greening and browning has varied widely across time and space due to differences in climatic variables across Alaska (Bhatt *et al.* 2017). The boreal forests have also experienced declines in growth in Interior Alaska (Juday *et al.* 2015), particularly in drier regions of the state (Beck *et al.* 2011).

Rising temperatures have accelerated significantly since 1998 at latitudes above than 59°N , causing strong shifts in the timing of life history events of plants and animals (Post *et al.* 2018). Insects are useful for inferring the impacts of climate change on the biosphere because

they have short generational turnover times compared to larger and longer living animals. Rising temperatures can accelerate the development rates of insects (Davidson 1944), causing some insects to emerge earlier in the warm season. Certain species of butterflies (Altermatt 2010) and spruce beetles (Temperli *et al.* 2015) possess flexible life history strategies enabling them to lay additional broods as climate warms. Early seasonal emergence can be advantageous for certain insects; for instance, the earlier emergence of mosquitoes in Greenland has led to greater synchronization with the seasonal appearance of their caribou hosts (Culler *et al.* 2015).

Changes in phenology, the timing of life-cycle events, can also lead to asynchrony between herbivorous insects and their hosts if insects emerge before plants have matured (Singer and Parmesan, 2010), and this can impact the pollination services some insects provide. Migratory birds who fly north to their summer breeding grounds in the Arctic may also miss the period of peak insect abundance and so find only limited food resources for their offspring (Thackeray *et al.* 2010). For the insects themselves, changing phenology can be detrimental if early emergence leaves the insect in a life stage that is unsuitable for winter survival (Van Dyck *et al.* 2015). At high latitudes, insects have adapted to short growing seasons (Danks, 1978), and their survival is critically dependent on having a suitable stage in which to overwinter (Avila-Jiménez *et al.* 2010). High-latitude insect species can withstand subzero temperatures during long winters only if they are in the correct life-history stage, but they must accumulate all their nutrients during a very short growing season so they are particularly sensitive to consequences of rising temperatures (Buckley *et al.* 2017).

Rising temperatures have been associated with dramatic changes in insect communities in the Arctic. At Zackenberg Research Station (74°28' N, 20°34' W) in Greenland, muscid flies (Diptera: Muscidae) monitored from 1996 to 2013 were found to have responded to rising

temperatures by declining in species diversity and population abundances (Loboda *et al.* 2018). This could be because increased temperatures have led to decreased soil moisture, which impacted the flies' fecundity (Loboda *et al.* 2018). In insects, exposure to heat stress can cause declines in fecundity, as measured by egg to adult viability and sperm motility (Porcelli *et al.* 2017). Declines in lifetime oviposition rates and egg viability, along with decreasing longevity, have been observed by Carroll and Quiring (1993) in caterpillars reared at high temperatures. Warming temperatures have been linked by Berger *et al.* (2008) to lowered fecundity in female butterflies because smaller body sizes exhibit commensurate reductions in brood size. Decreased body size due to warming temperatures has also been demonstrated in laboratory settings, most recently by Kennelly *et al.* (2017) in the common migratory butterfly *Vanessa cardui* (L. 1758). Declining size of butterflies reared at high temperature has been proposed by Jones *et al.* (1982) to result from increased metabolic costs that the caterpillars cannot compensate for.

Habitat warming has also had similar measurable effects in butterflies in Greenland. Butterflies (Lepidoptera: Papilionoidea) at Zackenberg Research Station have experienced climate-related changes, including a measurable decrease in wing size of two species of butterflies collected over a 17-year period (Bowden *et al.* 2015). Bowden *et al.* (2015) proposed that warming may cause reduced size because higher temperatures increase metabolism so much that larval feeding rates cannot keep up (Bowden *et al.* 2015). These caterpillars may be burning their on-board fuel faster than they can replace it. In contrast, the opposite trend of increased forewing length with rising temperatures was found in *Colias meadii* W. H. Edwards 1871 collected in the Rocky Mountains of Colorado (Maclean *et al.* 2016). These authors attributed this result to a lengthened growing season, which allowed for additional resource availability to compensate for the increased metabolic loss due to warming. These divergent morphological

responses of butterflies to warming temperatures in Greenland (Bowden *et al.* 2015) and Colorado (Maclean *et al.* 2016) warrant further investigation. Rising temperatures can impact insect development through the final size of adults and also through the timing of seasonal flight periods.

In Greenland, butterflies have similarly exhibited both a shift to earlier and shorter seasonal flight periods (Høye *et al.* 2014). Rising temperatures have been accompanied by earlier seasonal flights of butterflies in Manitoba, Canada (Westwood & Blair, 2010). Analysis of flight periods of Canadian butterfly species (Kharouba *et al.* 2014) highlighted the differences in the advancement of their flight periods due to ecological traits of individual species. Earlier seasonal flight, revealed by records from both natural history collections and also observational sightings of butterflies, has also been found in butterflies in Britain (Roy and Sparks, 2000), in the Mediterranean region (Stefanescu *et al.* 2003), and in the continental United States (Williams *et al.* 2014), indicating that there is a widespread effect across ecosystems and latitudes.

Rising temperatures can contribute additional stress on species under threat from other aspects of anthropogenic change. Declines in insect abundance during the last half-century across Europe (Biesmeijer *et al.* 2006, Hallmann *et al.* 2017) and North America (Cane *et al.* 2001, Kerr *et al.* 2015) have been attributed to multiple causes including large-scale habitat loss, pesticide applications, and light pollution (Potts *et al.* 2010). These stressors are likely to broadly affect arthropods, including taxa that are less conspicuous in scientific collections than butterflies.

Collections of biological organisms can be a valuable tool to quantify historical responses to environmental changes and to infer trends in species populations for well-represented insect groups. The insect research based at Zackenberg (Høye *et al.* 2014, Bowden *et al.* 2015, Loboda

et al. 2018) was made possible through long term (1996 to present) seasonal collections of specimens through the Greenland Ecosystem Monitoring Program whose specimens are housed at the Natural History Museum in Aarhus, Denmark. Insect collections are invaluable for investigating species-level impacts of warming at high latitudes (Sikes *et al.* 2017).

Butterfly specimens are ideal for climate change research because they are represented in museum collections made over time and across space (Seltmann *et al.* 2017). Their relatively large size is helpful in quantifying morphological impacts of rising temperatures, because measurements such as forewing length can be easily measured in millimeters with a set of calipers instead of more involved microscopic measurement required for smaller insect taxa. Along with morphological studies, collection data records that are associated with specimens are also useful for phenological studies inferring the timing of flight periods over multiple decades. Discerning patterns of species change over time has become increasingly attainable as natural history collections are made accessible through digitization (Johnson *et al.* 2011). Collections represent a baseline for comparisons over time, and in the Arctic a unique record exists for Alaskan butterflies from 1966 to 2014 through the Alaska Lepidoptera Survey (Sikes *et al.* 2017).

The Alaska Lepidoptera Survey, founded by the late Dr. Kenelm W. Philip, provided nets and data sheets to anyone who wanted to collect butterflies and moths. Dr. Philip's collection of Lepidoptera included over 111,800 specimens from Alaska, Canada, and Russia. The collection is currently held at the University of Alaska Museum in Fairbanks. Dr. Philip had over 600 volunteers who collected Lepidoptera for his Survey, which led to widespread but unequal coverage across geographic space and time. This study represents the first assessment of both morphological and phenological impacts of temperature on Alaskan butterflies, using

specimens and collecting records from the Alaska Lepidoptera Survey and the smaller Lepidoptera collection of the University of Alaska Museum.

Chapter 1: Wing length in Alaskan butterflies decreases during warmer summers across a broad geographic range

Abstract

Previous studies show that the body sizes of butterflies are influenced by the temperature experienced during larval development. It follows that warming climates should be expected to affect butterfly morphology. To test this prediction, I measured the forewing length of three species of holarctic butterflies, *Colias hecla* Lefebvre 1836 (n=581), *Boloria chariclea* (Edwards, 1883) (n=981), and *Boloria freija* (Thunberg, 1791) (n=939), collected in northern and northwestern tundra regions of Alaska between 1971 and 1995 in order to test whether spring and summer warming impacted butterfly morphology. Wing length was compared to growing degree days of both the summer of collection and the previous year's summer using mixed-effect statistical models. Results show that for every 1°C increase in average seasonal temperatures, wingspans of some of the Alaskan butterfly species studied decreased, between 0.16 millimeters (*Boloria chariclea* males) and 1.4 millimeters (*Boloria freija* males), surprisingly, and unlike in Greenland, *Colias hecla* did not exhibit any change in wing length in Alaska. Decreasing wingspans between 0.50 millimeters (in *Boloria chariclea* females) up to 0.64 millimeters (in *Colias hecla* females) had been previously reported in Greenland (Bowden *et al.* 2015). The magnitude of decreases in wing size may be dependent upon life-history traits of different species, with species overwintering as late-instar larvae (*Boloria freija* (Thunberg, 1791)) showing the largest decrease in wing length, and species overwintering as early-instar larvae (*Colias hecla* and *Boloria chariclea*) showing the least. Species overwintering as mid- to late instar larvae were most affected by changes in spring warmth, while species overwintering as eggs or neonate larvae were most affected by the previous summer's warmth.

Introduction

The temperature of the surrounding environment influences animal body size such that a trend exists for larger individuals to live in colder, more poleward habitats in both endothermic and ectothermic animals (Blackburn *et al.* 1999). This biogeographic pattern, known as *Bergmann's Rule*, asserts that endothermic animals are largest in the coldest environments because their larger size is more favorable for heat retention (Bergmann, 1847). However, this has only been documented in relatively few endothermic species, and the mechanisms of size-selection varies widely between taxa (Blackburn *et al.* 1999; Watt *et al.* 2010). Ectothermic animals can also show larger body sizes in colder climates, but the proposed mechanism differs: larger mass is selected for because of a related increase in fasting ability that increase survival during seasonal resource scarcity (Heinze *et al.* 2003). The *Temperature-Size Rule*, proposed by Atkinson (1994), also predicts larger body sizes in poleward ectotherm populations, but via an alternative mechanism, namely that lower temperatures reduce metabolism through a decrease in respiration relative to the rate of feeding. This lower metabolism relative to feeding rate promotes accumulation of nutrients in colder climates and so leads to faster growth.

Some ectothermic species exhibit striking local adaptations to regional temperature gradients. In a comparative study of Alaskan and Michigan populations of the swallowtail butterfly *Papilio canadensis* Rothschild & Jordan, 1906, Ayres and Scriber (1994) found that Alaskan larvae grew significantly faster at lower temperatures (12°C) than the Michigan populations, indicating the ability of different subpopulations of the same species to adapt to regional climates. Rising temperatures have been linked to rapid changes in butterfly body sizes, but species which have large geographic ranges may respond differently across their ranges.

The responses of butterfly species to warming varies between species. Forewing length of *Colias meadii* W. H. Edwards 1871, was found to increase in warmer environments at temperate latitudes in the Rocky Mountains (39.37°N, 106.18°W) of Colorado, USA (Maclean *et al.* 2016). Maclean *et al.* (2016) explained this trend by suggesting caterpillars had greater food availability due to host-plant warming-induced changes in plant phenology such that plants experienced prolonged growing seasons and so provided a prolonged season for caterpillars.

Climate warming has also been linked to declining wing length in two univoltine, Holarctic species of butterflies, *Colias hecla* Lefebvre 1836 and *Boloria chariclea* (Edwards, 1883) that were studied in Greenland over a 17-year period (Bowden *et al.* 2015). Warming-induced increases in larval metabolism could lead to a loss of nutrients that cannot be compensated for prior to pupation, leading to decreased adult body size (Bowden *et al.* 2015). Decreases in the sizes of adult butterflies raised under warming conditions have also been demonstrated in laboratory settings. Caterpillars of *Pieris rapae* (Linnaeus, 1758) reared at increased temperatures had reduced adult body sizes, indicating that if warming is severe, metabolism increases at a rate that cannot be compensated by increased food consumption (Jones *et al.*, 1982). Fecundity in insects, as measured by egg to adult viability and sperm motility, declines during exposure to heat stress (Porcelli *et al.* 2017). Spruce bud moth *Zeiraphera canadensis*, (Lepidoptera: Tortricidae), reared at constant high temperatures (20 - 25° C) exhibited declines in lifetime oviposition rates and egg viability, along with decreasing longevity (Carroll & Quiring 1993). In female butterflies of the speckled wood butterfly *Pararge aegeria* (Lepidoptera: Nymphalidae) high temperatures resulted in smaller adult body sizes and commensurate reductions in brood size (Berger *et al.* 2008). Warming-induced size decreases in

Lepidoptera could be a result of both a direct impact on their metabolism along with indirect impacts on the quality of their habitat.

It is also possible that warming effects on larvae are mediated through effects on host plants. For instance, rising temperatures can decrease plant productivity due to drought stress (Hatfield and Prueger, 2015). Also, elevated atmospheric CO₂ levels can lead to decreases in nutrient content (Rajashekar, 2018). Furthermore, increased CO₂ can alter the ability of plants to process nitrogen-containing compounds, leading to decreases in vitamins such as B1 (thiamine), B2 (riboflavin), B5 (pantothenic acid), and B9 (folate) along with decreases in minerals and trace elements (Zhu *et al.* 2018). Host-plant changes, coupled with other negative effects of climate change, such as habitat loss, habitat alteration, and increases in extreme weather events, are predicted to threaten many butterfly species, particularly those that are ecologically restricted (Settele *et al.* 2008).

Insect populations at the northern edges of their ranges in the Arctic may be particularly vulnerable to climate change (Strathdee & Bale, 1998). At Zackenberg Research Station in Greenland, the butterflies *Colias hecla* and *Boloria chariclea* exhibited reduced forewing length following the warmest years between 1996 – 2013 (Bowden *et al.* 2015). However, these specimens were collected at only a single site, and it is unclear if the response is generalizable to the entire Arctic. While much of the insect fauna of the Arctic is poorly sampled, a unique period of intense sampling of Alaskan butterfly fauna exists via the Alaska Lepidoptera Survey, which ran from 1966 – 2013.

The main objective of this study is to test whether the findings of Bowden *et al.* (2015) in Greenland can be corroborated in Alaska, which is located on the opposite side of the Arctic. Observing a similar relationship between wing size and temperature in Alaskan butterflies would

indicate the effect reported by Bowden *et al.* (2015) is likely acting on insects throughout the Arctic. A secondary objective is to test whether life-history traits are an important modifier of butterfly responses to warming climate. To accomplish these objectives, I compare the changing wing lengths of Alaskan populations of the same two species studied in Greenland, *Colias hecla* and *Boloria chariclea*, along with a third species, *Boloria freija*, which overwinters in an earlier larval stage and thus flies almost three weeks earlier in the season than the two other study species.

Methods

Specimens. I used butterfly specimens from the Kenelm W. Philip Lepidoptera Collection (henceforth the KWP collection) currently held at the University of Alaska Museum and in the process of being databased in Arctos, a publicly accessible museum collection management system (<http://arctos.database.museum/SpecimenSearch.cfm>). This collection spans from 1966 until 2014, and includes a total of 111,800 specimens from Alaska, Canada, and Russia. Due to a formal agreement between Dr. Philip and the Smithsonian Institution (SI), eventually 90% of the pinned KWP collection specimens (and the associated data) will be transferred to the SI and the associated data will not be accessible in Arctos. Specimens were collected by Dr. Philip and volunteers of his Alaska Lepidoptera Survey; a total of 2,501 specimens from the KWP collection were sampled for this study (Fig. 1).

Species Data. I studied *Colias hecla* and *Boloria chariclea* in order to make direct comparisons with the results reported from Greenland by Bowden *et al.* (2015). I also included *Boloria freija* in the study because it was the second most commonly collected species after *B. chariclea* within

the genus *Boloria* in northern Alaska. Most specimens were identified by Kenelm W. Philip; specimens not identified by KWP were identified by undergraduate students at the University of Alaska Fairbanks and confirmed by me. Because collecting effort across space and time for each species was not consistent, sample sizes of measured specimens between species and region were unequal (Table 1). I only included specimens still in their original glassine collecting envelopes. I deemed that pinned specimens were too fragile for students to measure safely.

Regional Breakdown. I selected butterflies collected in tundra regions in northern and western Alaska (Fig. 1). I chose to subdivide samples according to three geographic sub-regions: the Seward Peninsula, western North Slope, and eastern North Slope. This division was made to account for regional differences in both climate and collecting effort. On the North Slope, most specimens were collected from the eastern region because it is accessible by road. Consequently, the eastern North Slope was sampled more intensely and more consistently over time than the western region.

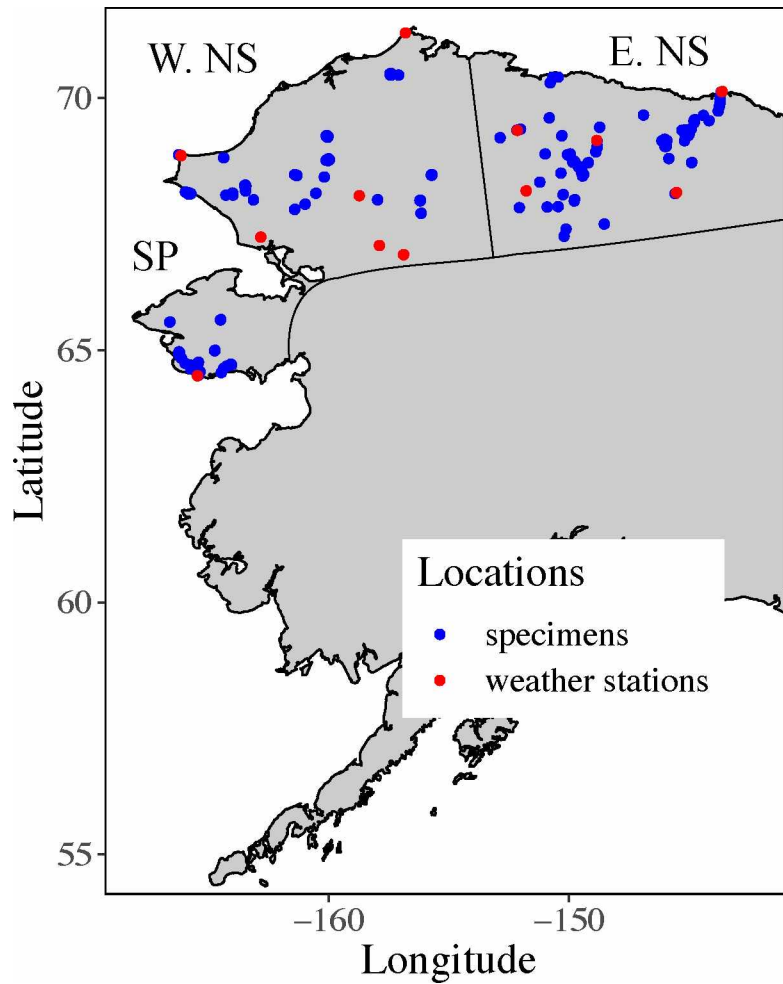


Figure 1: Map of localities where measured butterfly specimens were collected (blue) and weather stations (red) of Alaska, along with the region (SP = Seward Peninsula, W. NS= western North Slope, E. NS= eastern North Slope) which specimens were grouped in as a random effect in analysis. Plot made using ggplot2 (Wickham, 2016).

Table 1. Butterfly specimen sample size, including total measured individuals (n=2501) by species (rows) and region of Alaska (columns).

	Seward Peninsula	Western North Slope	Eastern North Slope	Total
<i>Colias hecla</i>	124	145	312	581
<i>Boloria chariclea</i>	249	299	433	981
<i>Boloria freija</i>	54	189	696	939
total	427	633	1441	2501

Specimen measurement. Measurements were performed by a group of 15 undergraduate students at the University of Alaska Fairbanks. A reference set of 11 specimens were measured by all students to assess differences in measurements attributable to variation among students. Measurements were compared to the known values for wing length using a one-way ANOVA to detect any significant differences among students and, when found, students were reassessed after further instruction until differences in measurements among students were not detectable.

Each specimen envelope was placed on a light box to illuminate wing and thorax shape. While still in the envelope, students measured forewing length from wing apex to thorax segment to the closest 0.1 mm using digital calipers. To minimize handling damage, I chose not to have the students measure wings using the conventional measurement of apex to thoracic attachment. Specimens were sexed to account for sexual size dimorphism. To do this, dorsal wing patterns were used for *C. hecla* as they are clearly sexually dimorphic in color patterns, whereas *B. chariclea*, and *B. freija* were sexed by examination of genitalia under magnification.

Temperature data. Alaska minimum and maximum daily temperature data for 1970 – 1995 were obtained from the Global Historical Climatology Network for twelve weather stations (Fig. 1) (<https://www.ncdc.noaa.gov/data-access>). To estimate temperature conditions at the time of specimen collection, specimens were associated with the closest active weather station to their

collection site (Fig. 2). I used Google Earth to determine the closest station by creating Keyhole Markup Language (KML) file overlays using the geo-coordinates of weather stations and specimen collection events.

Standardization of temperature data. The Seward Peninsula had consistent collection of temperature data (Nome station), but the eastern and western North Slope regions had inconsistent sampling histories; daily temperature records were occasionally erroneous or absent for inland stations of both regions. Continuous temperature records were available for coastal weather stations for the majority of the sample period (1970-1988) in both regions (Fig. 2), but many butterflies were collected in inland areas that were far from any operating weather station (Fig. 1).

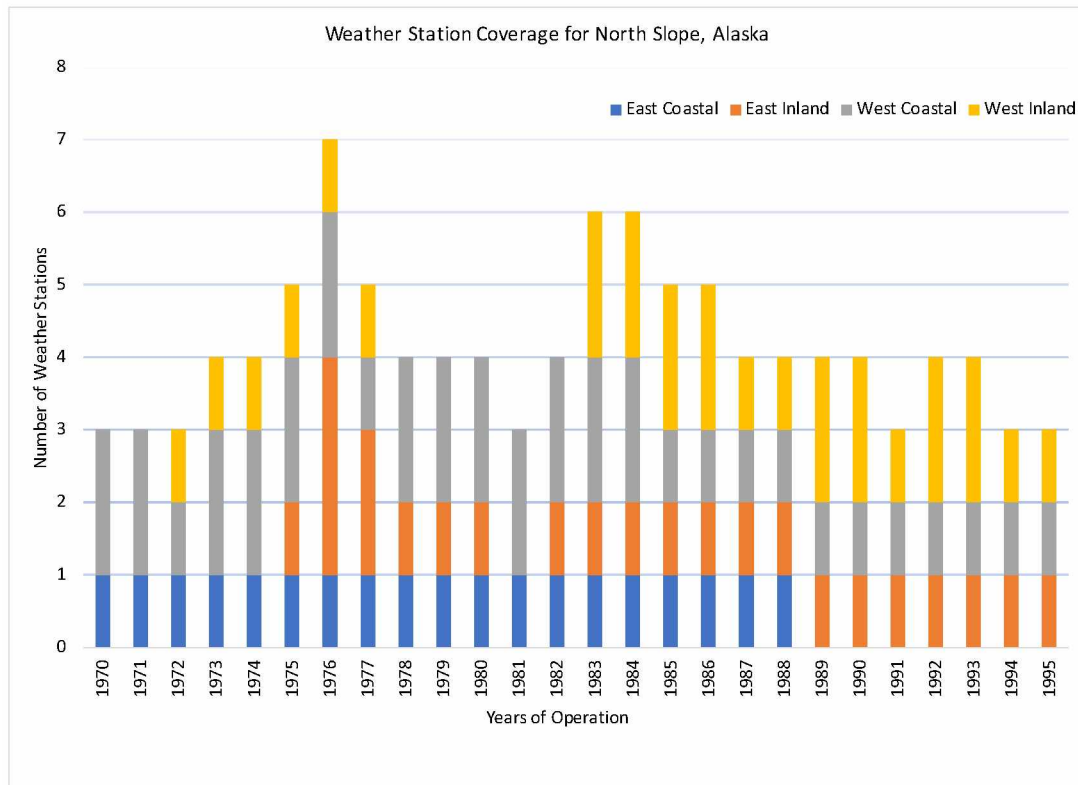


Figure 2. Alaskan weather stations in operation on the North Slope between 1970 and 1995, separated by the regions depicted in Fig. 1 showing continuity of coverage between coastal eastern stations (blue), inland eastern stations (orange), and coastal western stations (grey), and inland western stations (yellow)

Because coastal areas are typically cooler than inland ones, temperature records are problematic in years where no inland weather station was recording (Fig. 2). Coastal and inland temperatures on the North Slope of Alaska are markedly different across the summer growing season, with higher summer temperatures occurring inland and cooler temperatures near the coasts (Figs 3 & 4). Temperature patterns also differ between the eastern and western regions of the North Slope of Alaska. The eastern North Slope of Alaska shows relatively synchronized seasonality across the region; inland and coastal stations begin to warm at roughly the same time in spring. However, at the peak of summer, it can be almost 10°C warmer inland than along the coast in the eastern North Slope. In the winter, coastal stations there are warmer due to an inversion effect that occurs inland (Fig. 3). The Western North Slope also showed marked seasonality differences between coastal and inland sites. Inland, spring temperatures begin to rise earlier in the season than coastal stations, and consistently higher temperatures are recorded inland throughout almost the entire year (Fig. 4).

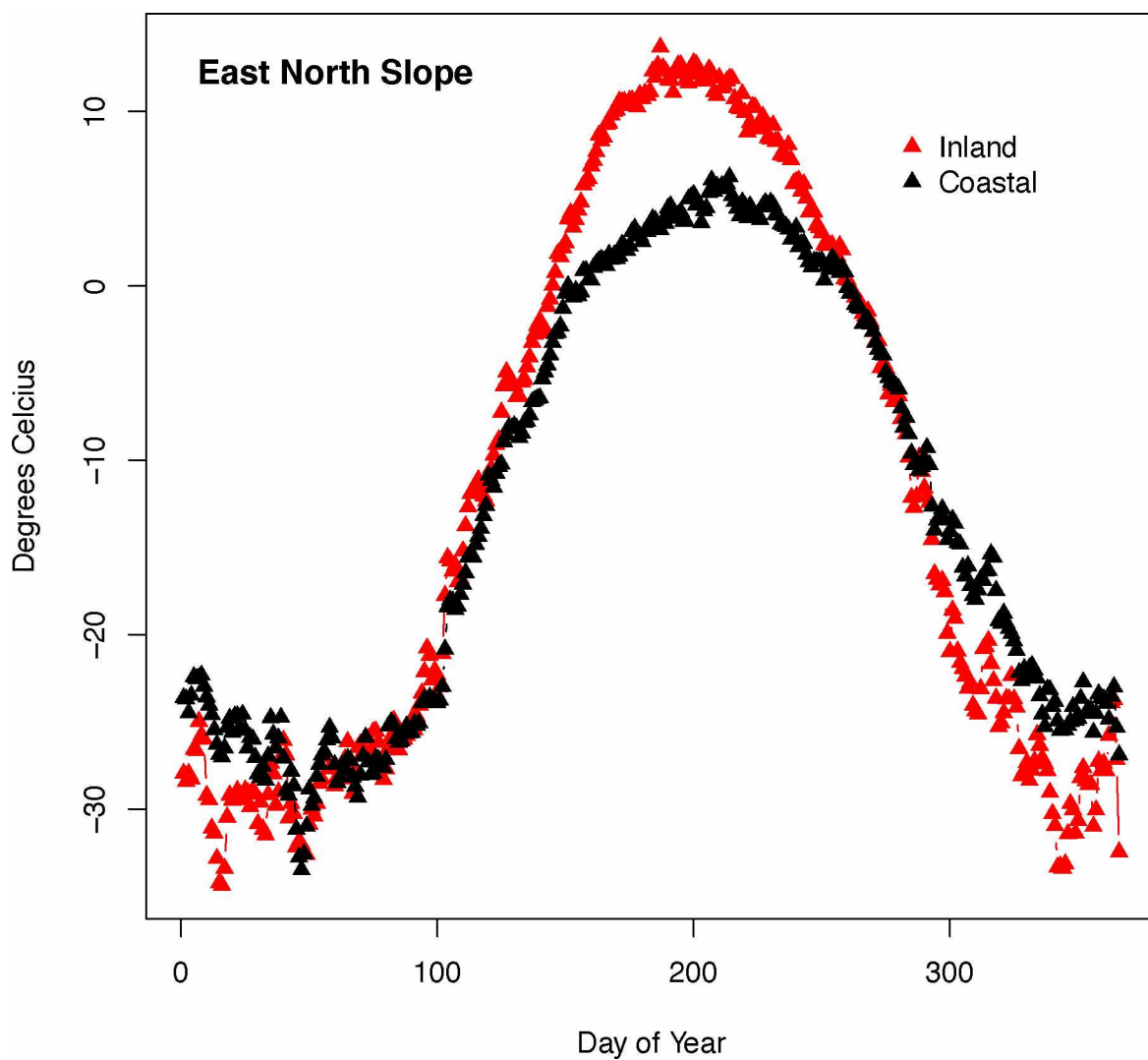


Figure 3. Mean daily temperature (°C) for Alaskan eastern North Slope inland (red) and coastal weather stations (black) between 1970 – 1995, located between 155°W and 142°W longitude.

To resolve the missing temperature data from inland stations in each region, I created a correction for estimating inland temperature for any given year using the coastal observations. Given the known mean daily temperatures for inland and coastal areas of each region (depicted in Figs 3 & 4), I calculated the average daily difference between coastal and inland temperatures using all available years when data were available for both inland and coastal sites. I then calculated an estimated temperature for inland sites for years when inland data were missing by adding the mean daily difference to the measured coastal temperature for any given daily observation across years when there was no inland data.

After building the temperature records, I calculated cumulative growing degree days (GDD), an additive calculation of temperature above a certain threshold (Gordon & Bootsma, 1993). GDD is a direct measure for total heat accumulated at a given location, and I used it to quantify the heat experienced during butterfly development during both the spring of the year specimens were collected as well as the previous summer. Spring GDD and Summer GDD were treated as separate variables. Because the species studied are cold adapted, I used a threshold of 5° C. All daily temperatures surpluses above 5° C were thus added together to obtain cumulative growing degree days for every day for each station. Additionally, for years with missing interior site temperature data, the same GDD procedure was done, except using the estimated temperature per the method previously described using available coastal temperature data from either Barrow (Western North Slope) or Barter Island (Eastern North Slope) to estimate interior temperatures.

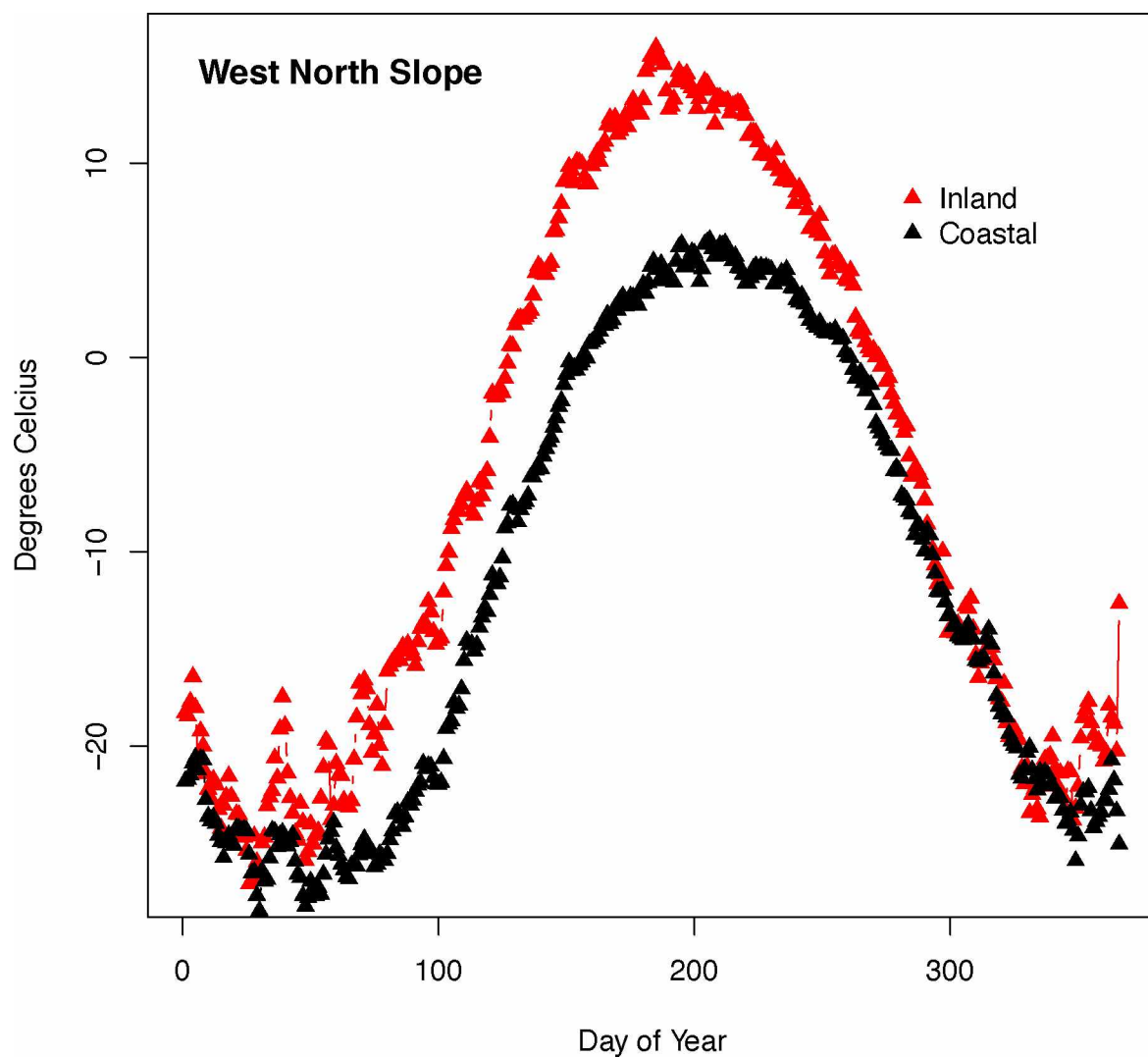


Figure 4. Mean daily temperature (°C) for Alaskan western North Slope inland (red) and coastal weather stations (black) between 1970 – 1995, located between 167°W and 155°W longitude

I used flight period data from the specimen records (Fig. 5) to estimate approximately when larval development was occurring in each species. This was necessary to constrain the calculation of accumulated GDD so that it only encompassed the larval development period (pre-flight period) for each species. For *Boloria freija*, the average day of year which species flight begins is approximately day 160, while for *Boloria chariclea* and *Colias hecla* this is day 180. Those dates then served as a cut-off for accumulated GDD values. To represent the likely temperature experienced during the developmental phase by the larval and pupal stages during spring of the flight year, I used growing degree day values calculated *up to* the mean first flight date for each species. I also calculated GDD *after* the mean first flight date for the previous year to represent heat experienced by the egg and early-to-late larval stage, thus initiating GDD at day 161 or 181, depending on species.

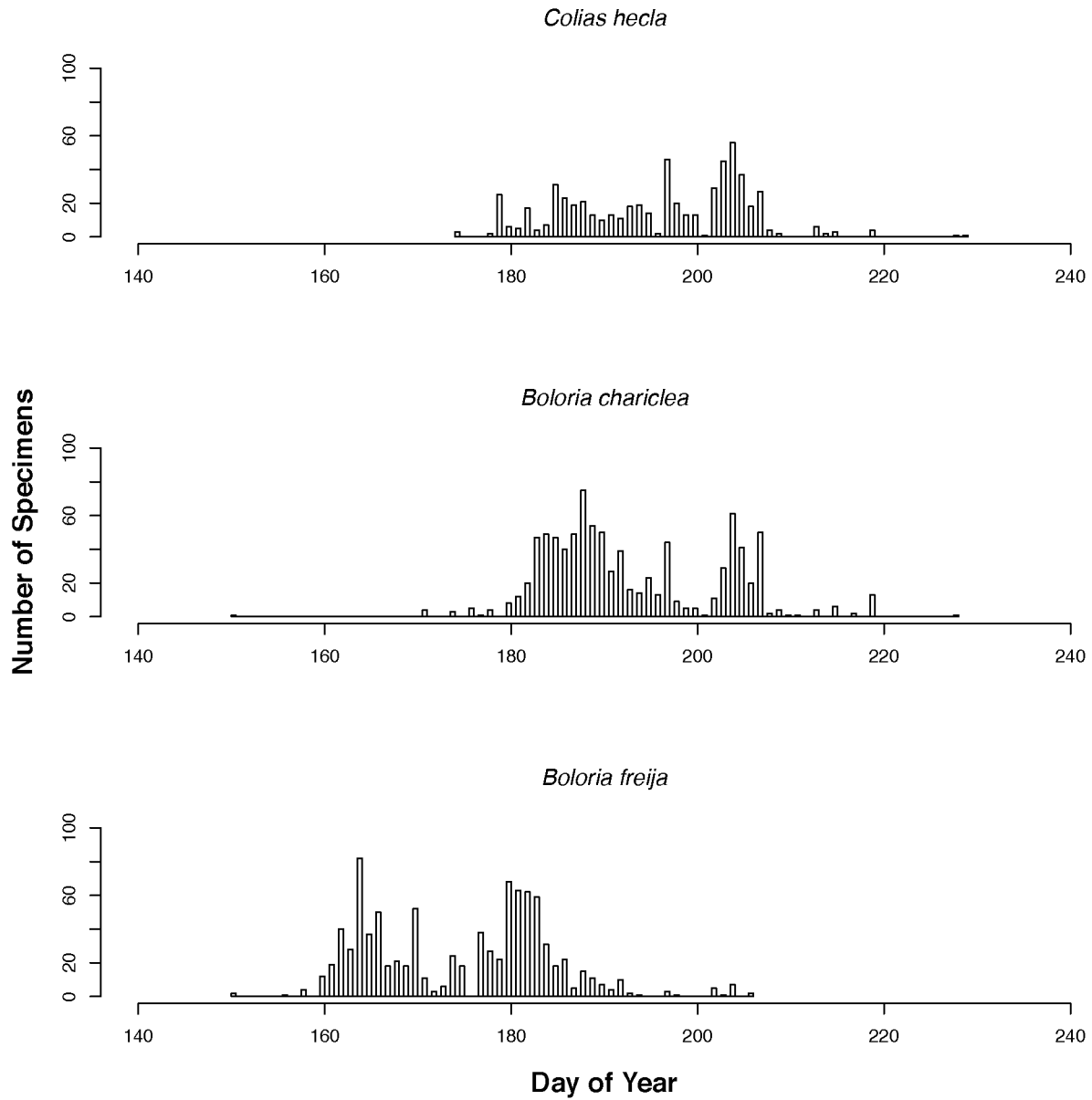


Figure 5. Seasonal collection records combined for the Seward Peninsula and both the eastern and western North Slope for *Colias hecla* (top) *Boloria chariclea* (middle) and *Boloria freija* (bottom) by Day of Year (DOY). The first flights of both *Colias hecla* and *Boloria chariclea* began around DOY 180 (29 June), whereas the majority of *Boloria freija* were collected after DOY 160 (9 June) from 1971 – 1995.

Statistical analysis. I used R version 3.4.3 “Kite-Eating Tree” (R Core Team, 2018) to analyze the data with the mixed effects model packages “nlme” (Pinheiro *et al.* 2016), and “lme4” (Bates *et al.* 2015). I created mixed-effects models with a random effect of region (because individuals from some regions are larger than others) and tested wing length as a response variable with the explanatory, fixed effects of sex, species, and spring and/or previous summer GDD data. The null model excluded GDD data and included only additive explanatory variables of species and sex with region as a random effect. These models were created to test specific hypotheses about how temperature might be influencing size and how demographic and life history traits such as sex and overwintering state might influence temperature effect on morphology. Thus, models with specific combinations of additive main effects and interactions between sex, species, and GDD data for each season were tested and explanatory power assessed using the Akaike Information Criterion (AIC) for model selection (Table 2).

Results

Model Selection. The best-fitting model explaining wing length included “sex” nested within “species”, and interactions between seasonal temperatures, including both spring GDD of the collecting event year (later instar / pupa) and the prior year’s summer (egg, early instar caterpillar). Generally, wing length decreased as temperature increased, a relationship best explained by temperature interactions experienced during spring and the previous summer between both species and sex (“Species/sex * Previous summer GDD + Species/sex * Spring GDD”, $df=17$). The fact that the best model had the fixed effects of sex nested within species (accounting for sexual dimorphism within a species) indicates that the responses of males and females of a given species to warming are not independent. I present the following model-

selection results as changes in AIC scores (Table 2), with the lowest score indicating the best-fitting model to explain wing length.

The next best model included sex and species only as an interaction (tested as “Sex * Species * Summer + Sex * Species * Spring”: Δ AIC +7.2), although I found that including sex only as an additive explanatory variable to wing length (tested as Sex + Species * Summer GDD + Species * Spring GDD) offered a slight decrease in model fit (Δ AIC +8.3).

Table 2: Comparison of linear mixed effect models testing butterfly wing length as a function of sex, species, and spring and/or previous summer GDD values, with Akaike Information Criterion (AIC) score depicted as change from the best fitting (lowest scoring) model. GDD data were not included in null model, but all models included a random effect of region

Model	DF	AIC Score	Δ AIC
Length ~ Species / Sex * Summer GDD + Species / Sex * Spring GDD	17	7530.2	0
Length ~ Sex * Species * Summer + Sex * Species * Spring	14	7537.4	+7.2
Length ~ Sex + Species * Summer GDD + Species * Spring GDD	12	7538.5	+8.3
Length ~ Sex + Species + Summer GDD + Spring GDD	8	7563.5	+33.3
Length ~ Sex + Species * Summer GDD	9	7563.9	+33.7
Length ~ Sex + Species + Summer GDD	7	7570.3	+40.1
Length ~ Sex + Species * Spring GDD	9	7574.8	+44.5
Length ~ Sex + Species + Spring GDD	7	7586.0	+55.8
Length ~ Sex + Species (null)	6	7636.4	+106.2

An additive model including sex, species, and both spring and summer temperature data (“Length ~ Sex + Species + Summer GDD + Spring GDD”: Δ AIC +33.323) was a better fit than using either season’s temperature data alone (“Sex + Species * Summer GDD” or “Sex + Species * Spring GDD”).

Though both stages of seasonal development are important, temperatures experienced during the previous summer (during early development) appear to influence wing-length development more than the temperatures experienced during later development in the spring when adults emerged. This finding was further tested using summer temperature as both an interactive effect on species (“Sex + Species * Summer GDD”: Δ AIC +33.76) or simply an additive effect (“Sex + Species + Summer GDD”: Δ AIC +40.09). Summer-only models tended to perform better than the corresponding models that included only spring temperature data. Spring-only models performed worse when spring GDD was tested as an interaction (“Sex + Species * Spring GDD”: Δ AIC +44.567) or as an additive effect (“Sex + Species + Spring GDD”: Δ AIC +55.838). This result reinforces the importance of temperatures experienced during early larval development in the summer. The null model had the highest AIC score (“Sex + Species”: Δ AIC +106.221) compared to the best fitting model, indicating that seasonal temperature does explain wing length.

Colias hecla, *Boloria chariclea*, and *Boloria freija* show divergent morphological responses to seasonal temperatures. In summarizing the best model (“Species / sex * Previous summer GDD + Species / sex * Spring GDD”), spring GDD is non-significant as a main effect ($p=0.9242$), because species and sexes were differentially affected by spring warming, and interactions between species and temperature and sex and temperature were highly significant. This indicates some species and some sexes (usually males) were more affected than others. I report these divergent results next. To present results across species, I quantify wingspan size decreases per 100 GDDs. For comparison between Alaska and Greenland, I also present the effect size per 133 GDDs, which is comparable to a 1° C increase in average summer (May-August) temperature. I calculated this using Nome temperature records and first determined the

difference between average and warm summer GDD values (a difference of 213 GDDs) across the study period (1971-1995) using May – August records. Then, I found the difference between temperature in an average to a warm summer, an increase of 1.6 °C. Using this ratio, I calculated that an increase of 0.752 °C had occurred for every 100 GDDs, so for 1° C, an increase of about 133 GDDs occurred. I present the temperature effect on wingspan by multiplying the estimated forewing effect (shown in figures 6-8) by two.

***Boloria freija*.** *Boloria freija* males exhibited the strongest negative wing length response to increasing temperature; they were sensitive to increasing GDDs in both the spring of their adult year ($p < 0.0001$) and the previous summer ($p < 0.0001$). *B. freija* males exhibited an average forewing decrease of 0.535 millimeters \pm 0.112 SE per 100 GDDs experienced in the spring (Fig. 6, right column). This effect, multiplied by two, equates to a wingspan decrease of -1.071 mm \pm SE 0.224 per 100 GDDs. For every 1° C increase in average spring temperatures, *B. freija* males exhibited a wingspan decrease of -1.423 mm \pm SE 0.298. However, female *B. freija* did not show a significant change in wing length attributed to spring warming ($p = 0.6659$). In summer, when *B. freija* does the majority of its growing, males exhibited -0.139 mm \pm SE 0.026 decrease per 100 GDD's of accumulated warming whereas females exhibited a decrease of -0.113 mm \pm SE 0.047 (Fig. 6, left column). A 1° C increase from average summer temperatures was associated with a decreased wingspan of -0.185 mm \pm SE 0.035 for male *B. freija* and -0.151 mm \pm SE 0.062 for female *B. freija*.

Boloria freija

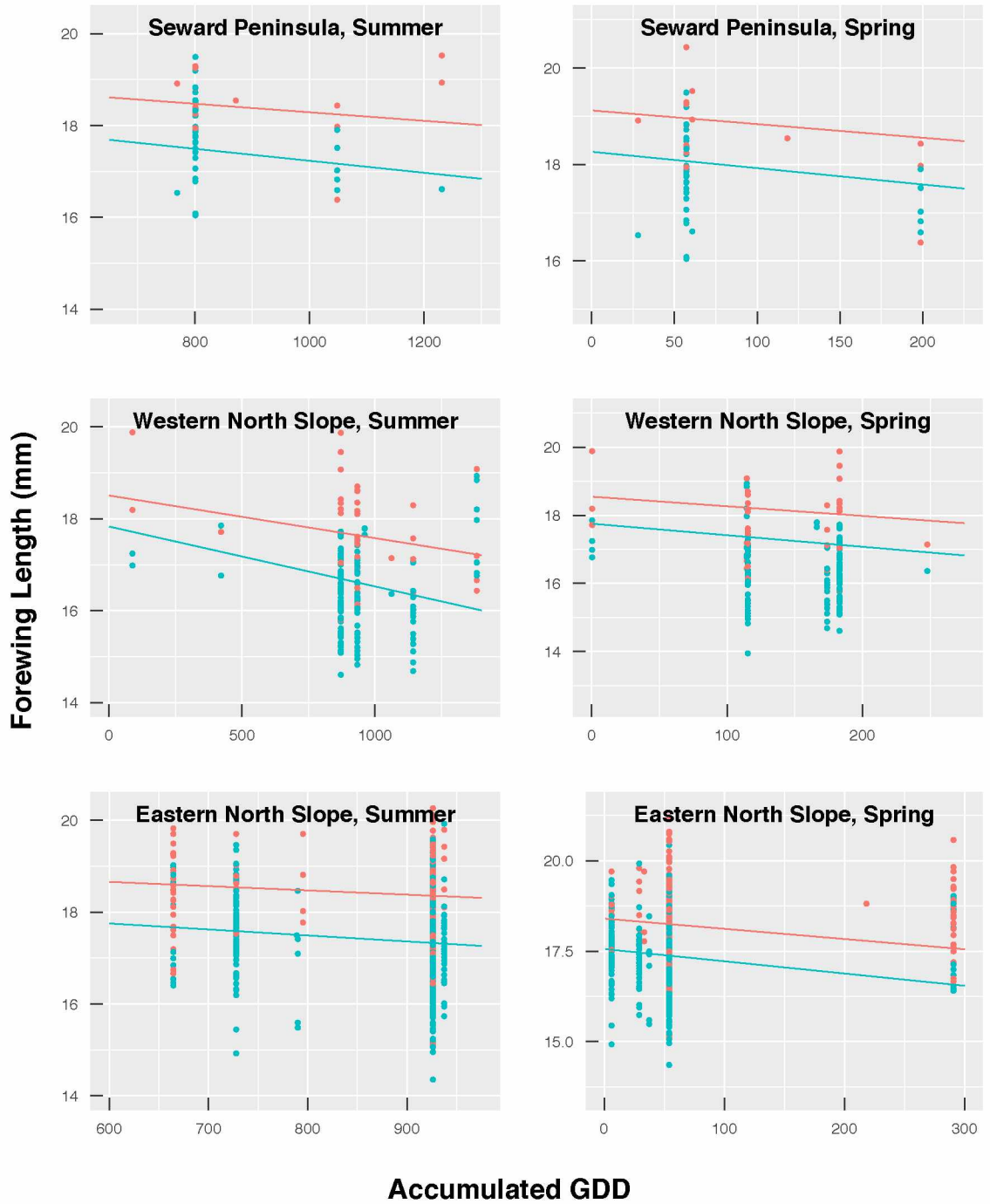


Figure 6. *Boloria freija* forewing length compared with growing degree days (GDD) during the previous summer (left column) and during the spring of collection year (right column) for females (pink) and males (blue) on the Seward Peninsula (top), western North Slope (center), and eastern North Slope (bottom). Plot made using ggplot2 (Wickham, 2016).

Boloria chariclea

Unlike *Boloria freija*, *B. chariclea* males had a non-significant ($p = 0.078$) decrease in wingspan attributable to spring warming (Fig. 7, right column). *Boloria chariclea* females were not affected by spring warming ($p=0.924$). However, summer warming (Fig. 7, left column) experienced by *B. chariclea* females was associated with significant ($p < 0.05$) decreased wing length of $0.157 \text{ mm} \pm 0.071 \text{ SE}$ per 100 GDDs, or a wingspan decrease of $-0.208 \text{ mm} \pm 0.047 \text{ SE}$ per 1°C increase from average temperatures. *B. chariclea* males were significantly affected by summer warming ($p = 0.037$) with a wingspan decrease of $-0.124 \text{ mm} \pm 0.029 \text{ SE}$ per 100 GDDs (Fig. 7b). A 1°C increase in average summer temperatures (133 GDDs) was associated with a decreased wingspan of $-0.165 \text{ mm} \pm 0.079 \text{ SE}$ in *B. chariclea* males.

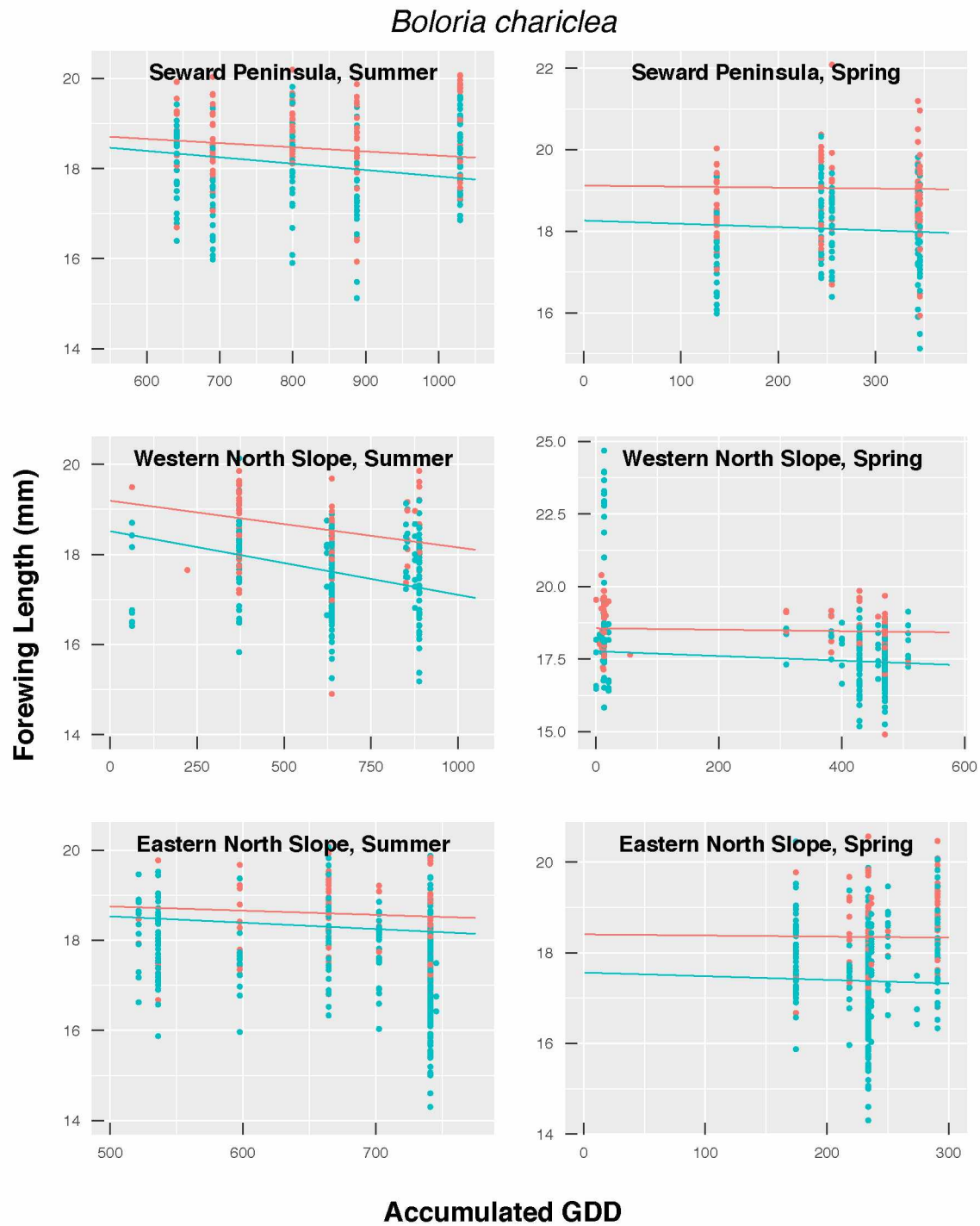


Figure 7. *Boloria chariclea* forewing measurements compared with growing degree days during the previous summer (left column) and during the spring of collection year (right column) for females (pink) and males (blue) on the Seward Peninsula (top), western North Slope (center), and eastern North Slope (bottom). Plot made using ggplot2 (Wickham, 2016).

Colias hecla

Spring warming did not accompany a significant trend in wingspan for either *Colias hecla* males ($p=0.389$) or *C. hecla* females ($p=0.556$) (Fig. 8, right column). Summer warming also did not have a significant effect on *C. hecla* females ($p=0.186$) or *C. hecla* males ($p=0.183$) (Fig. 8, left column).

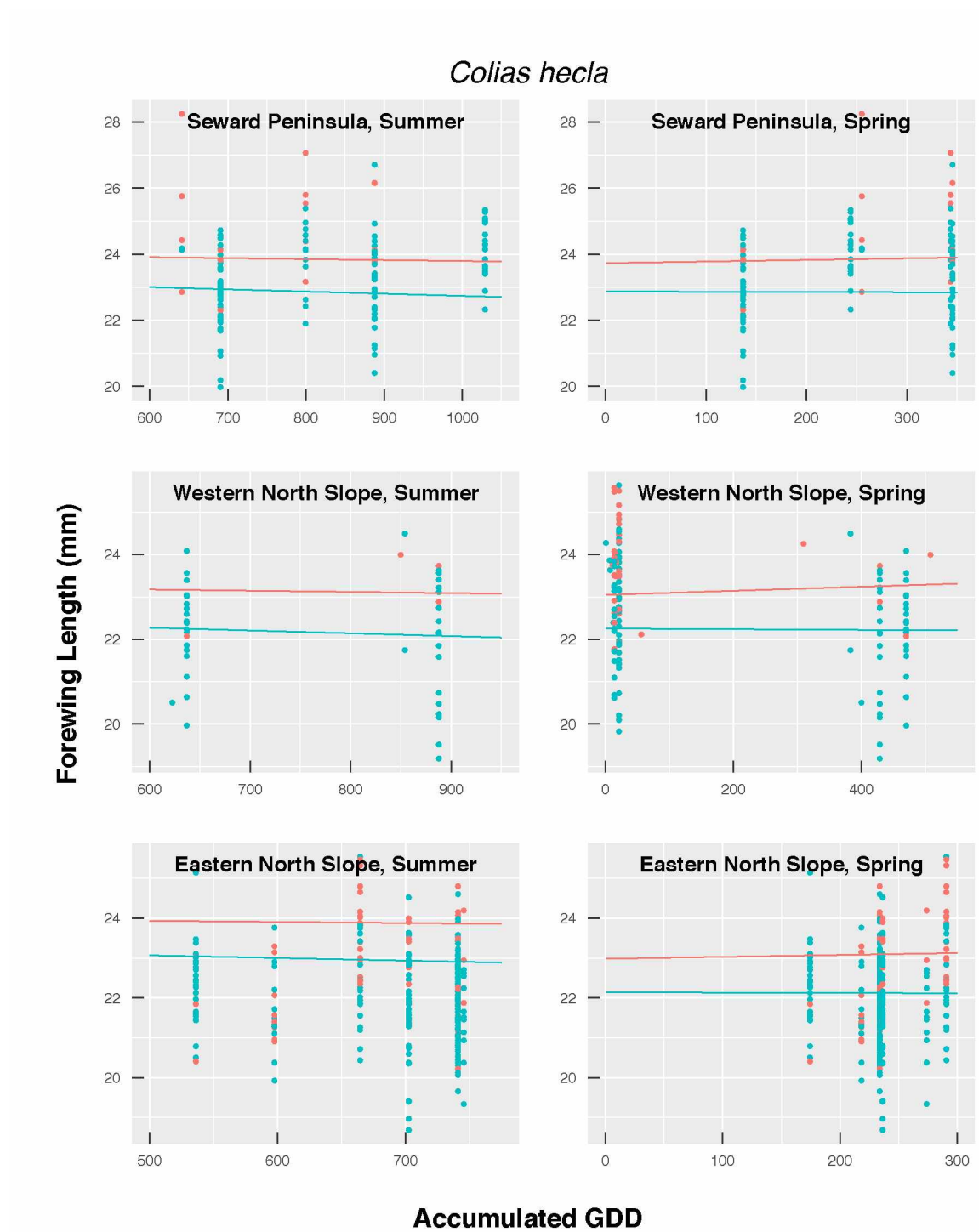


Figure 8. *Colias hecla* forewing length measurements compared with growing degree days (GDD) during the previous summer (left column) and during the spring of collection year (right column) for females (pink) and males (blue) on the Seward Peninsula (top), western North Slope (center), and eastern North Slope (bottom). Plot made using ggplot2 (Wickham, 2016).

Discussion

These results both corroborate and add a considerable amount of nuance to the recent similar findings from Greenland (Bowden *et al.* 2015). The effect of impacts of warming temperature on butterfly morphology is an Arctic-wide phenomenon, but it also has a complex nature. Unlike the two similarly responding species in Greenland, changes in wing length differed among the three Alaskan species and between sexes, with males tending to be more sensitive than females. In contrast, Bowden *et al.* (2015) found that for every 1°C increase in summer temperatures, *Boloria chariclea* and *Colias hecla* wingspans decreased between 0.50 and 0.64 millimeters, and they found no difference in the temperature effect between males and females.

Differences in life history traits among the three Alaskan species, particularly how and at what stage they overwintered, may explain the divergent responses to warming temperatures. Of the three species measured, *Boloria freija* has the earliest flight period (Fig. 4) and overwinters as a fourth-instar larva (Layberry *et al.* 1998). Warming spring temperatures could lead to a reduction in forewing length if unusual warmth causes metabolic stress in late-instar larvae that cannot be compensated for by additional feeding before pupation. When reared in captivity, *Boloria freija* was found not to feed during its fifth and final instar (James *et al.* 2011). As development during the 5th instar relies completely on fat reserves, warming spring temperatures may have a higher impact because additional metabolic expenditure cannot be compensated through additional feeding.

Boloria freija's inflexible diapause requirements may contribute to this species' sensitivity to spring warming. James *et al.* (2011) attempted to artificially break winter dormancy in *B. freija* by exposure to artificially warm temperatures in order to induce pupation early, but

was unsuccessful; larvae remained in the 4th instar without resuming activity or initiating pupation. These authors note that *B. freija* may be vulnerable to climate change because of its need for a long uninterrupted period (4-7 months) of cold temperatures prior to breaking winter diapause. Because winter temperatures in Arctic Alaska are continuously below freezing, this issue is more likely to affect individuals at southern range margins (Breed *et al.* 2013).

Timing of life cycles may explain the divergent responses between sexes of *B. freija* and the closely related *B. chariclea*. Male butterflies of many species are often found flying earlier in the season than females (Iwasa *et al.* 1983, Wiklund & Fagerström 1977), so it's possible that males may show greater morphological response to warming temperatures because development occurs slightly earlier than females of the same species.

Morphological sensitivity to warming appears to occur throughout the range of the pan-Arctic butterfly species *Boloria chariclea*. In Greenland, *Boloria chariclea* showed a negative trend of -0.31 mm forewing length for every 1°C increase in average summer temperatures (Bowden *et al.* 2015), or a wingspan decrease of -0.62 mm per degree. In Alaska, *B. chariclea* wingspan decreased 0.165 mm \pm 0.079 SE for every 1°C increase from average previous summer temperatures. Bowden *et al.* (2015) collected all of the Greenland specimens from a single locality, whereas the Alaskan specimens analyzed here were collected from a number of different localities between 64° 30' (Nome) to 71°17' N (Point Barrow).

The warming effect on the sizes of Alaskan *C. hecla* were not significant, which is different from the species' response in Greenland and from the responses of *B. chariclea* and *B. freija* in Alaska. A possible explanation is that Alaskan populations of *C. hecla* are more plastic in the duration of their overwintering stage, which could afford flexibility in adapting to rising temperatures. Research performed by Harry (2009) indicates that *C. hecla* on Alaska's North

Slope overwinter at a range of larval ages, including first, second, and third instar larvae. Some individuals remain in first instar to overwinter even if the seasonal timing of warmth and host plants would have allowed for additional molts before winter. It is possible that this developmental flexibility could help mitigate the effects of seasonal warming.

The divergent responses of *Boloria freija*, *Boloria chariclea*, and *Colias hecla* indicate that species' life histories are important determinants of a butterfly's adaptability in a warming world. Metabolic stress, thought to be the mechanism for the morphological responses observed in Greenland (Bowden et al, 2015), could lead to higher respiration and lower growth rate among Arctic Lepidoptera (Barrio et al 2016). Warming may favor butterfly species that are able to spread development evenly across two seasons (e.g. *B. chariclea* & *C. hecla*) compared to species that are required to overwinter as older caterpillars (*B. freija*) and so are less able to compensate for warming-induced metabolic stress with additional feeding in the spring. It follows that it is important to not only investigate body-size trends across space and time as climate changes in the Arctic, but also to account for differences in species' life histories.

Decreasing body size as a response to warming has recently been reported in other insect groups outside the Arctic, including ground beetles (Coleoptera: Carabidae) (Tseng *et al.* 2018) and a species of bee, *Osmia ribifloris* (Hymenoptera: Megachillidae), which was measured as part of a manipulative warming experiment (CaraDonna *et al.* 2018). Butterflies, beetles, and bees are some of the most visible of insect groups, but the species currently represented in morphology research are only a tiny fraction of the terrestrial arthropods undergoing severe habitat warming. These shared responses are important because they could be a harbinger for widespread changes occurring across many insect taxa.

Literature Cited

- Atkinson, D. (1994). Temperature and organism size: a biological law for ectotherms? *Advances in Ecological Research*, 25(1): 1-58.
- Ayres, M. P., & Scriber, J. M. (1994). Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). *Ecological Monographs*, 64(4), 465-482.
- Barrio, I. C., Bueno, C. G., & Hik, D. S. (2016). Warming the tundra: reciprocal responses of invertebrate herbivores and plants. *Oikos*, 125(1): 20-28.
- Berger, D., Walters, R., & Gotthard, K. (2008). What limits insect fecundity? Body size-and temperature-dependent egg maturation and oviposition in a butterfly. *Functional Ecology*, 22(3): 523-529.
- Bergmann, C. (1847). Ueber die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. Gottinger studien, [translation: On the relations of the heat economy of the animals to their size. Gottinger studies] 3, 595–708.
- Blackburn, T. M., Gaston, K. J., & Loder, N. (1999). Geographic gradients in body size: a clarification of Bergmann's rule. *Diversity and Distributions*, 5(4): 165-174.
- Bowden, J. J., Eskildsen, A., Hansen, R. R., Olsen, K., Kurle, C. M., & Høye, T. T. (2015). High-Arctic butterflies become smaller with rising temperatures. *Biology Letters*, 11(10): 1-5
- Breed, G. A., Stichter, S., & Crone, E. E. (2013). Climate-driven changes in northeastern US butterfly communities. *Nature Climate Change*, 3(2): 142-145

- CaraDonna, P. J., Cunningham, J. L., & Iler, A. M. (2018). Experimental warming in the field delays phenology and reduces body mass, fat content and survival: Implications for the persistence of a pollinator under climate change. *Functional Ecology*, 32(10): 2345-2356
- Carroll, A. L., & Quiring, D. T. (1993). Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera canadensis*. *Oecologia*, 93(2), 233-241.
- Gordon, R., & Bootsma, A. (1993). Analyses of growing degree-days for agriculture in Atlantic Canada. *Climate Research*, 169-176.
- Harry, J. L. (2009). Natural Life Histories of Alaska Colias (Lepidoptera: Pieridae). *The Taxonomic Report of the International Lepidoptera Survey*, 7(2): 1-22.
- Hatfield, J. L., & Prueger, J. H. (2015). Temperature extremes: effect on plant growth and development. *Weather and Climate Extremes*, 10(1): 4-10.
- Heinze, J., Foitzik, S., Fischer, B., Wanke, T., & Kipyatkov, V. E. (2003). The significance of latitudinal variation in body size in a holarctic ant, *Leptothorax acervorum*. *Ecography*, 26(3): 349-355.
- Høye, T. T., Eskildsen, A., Hansen, R. R., Bowden, J. J., Schmidt, N. M., & Kissling, W. D. (2014). Phenology of high-arctic butterflies and their floral resources: species-specific responses to climate change. *Current Zoology*, 60(2): 243-251.
- Iwasa, Y., Odendaal, F. J., Murphy, D. D., Ehrlich, P. R., & Launer, A. E. (1983). Emergence patterns in male butterflies: a hypothesis and a test. *Theoretical Population Biology*, 23(3): 363-379.
- James, D. G., Nunnallee, D., & Pyle, R. M. (2011). Life histories of Cascadia butterflies. *Corvallis: Oregon State University Press*. (p. 447).

- Jones, R. E., Hart, J. R., & Bull, G. D. (1982). Temperature, size and egg production in the cabbage butterfly, *Pieris rapae*. *Australian Journal of Zoology*, 30(2): 223-231.
- Layberry, R. A., Hall, P. W., & Lafontaine, J. D. (1998). The butterflies of Canada. *University of Toronto Press*. (p. 354).
- MacLean, H. J., Kingsolver, J. G., & Buckley, L. B. (2016). Historical changes in thermoregulatory traits of alpine butterflies reveal complex ecological and evolutionary responses to recent climate change. *Climate Change Responses*, 3(1): 13.
- Porcelli, D., Gaston, K. J., Butlin, R. K., & Snook, R. R. (2017). Local adaptation of reproductive performance during thermal stress. *Journal of Evolutionary Biology*, 30(2): 422-429.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rajashekar, C. B. (2018). Elevated CO2 Levels Affect Phytochemicals and Nutritional Quality of Food Crops. *American Journal of Plant Sciences*, 9(2): 150-162.
- Settele, J., Kudrna, O., Harpke, A., Kühn, I., Van Swaay, C., Verovnik, R., & Kühn, E. (2008). Climatic risk atlas of European butterflies Sofia-Moscow: *Pensoft*. (p. 710).
- Strathdee, A. T., & Bale, J. S. (1998). Life on the edge: insect ecology in arctic environments. *Annual Review of Entomology*, 43(1): 85-106.
- Tseng, M., Kaur, K.M., Soleimani Pari, S., Sarai, K., Chan, D., Yao, C.H., Porto, P., Toor, A., Toor, H.S. and Fograscher, K., (2018). Decreases in beetle body size linked to climate change and warming temperatures. *Journal of Animal Ecology*, 87(3): 647-659.
- Watt, C., Mitchell, S., & Salewski, V. (2010). Bergmann's rule; a concept cluster? *Oikos*, 119(1): 89-100.

- Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
- Wiklund, C., & Fagerström, T. (1977). Why do males emerge before females? *Oecologia*, 31(2): 153-158.
- Zhu, C., Kobayashi, K., Loladze, I., Zhu, J., Jiang, Q., Xu, X., Liu, G., Seneweera, S., Ebi, K.L., Drewnowski, A. and Fukagawa, N.K., (2018). Carbon dioxide levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. *Science Advances*, 4(5): 10-12.

Chapter 2: Phenological advancement of the early-season flights of Interior Alaska butterfly species, 1966 to 2016

Abstract

Seasonal advancements of insect flight periods have been documented to accompany rising air temperatures in diverse ecosystems. In polar regions where warming is most rapid, some species of Lepidoptera have advanced the timing of their first spring flights by several days per decade. Growing seasons at high latitudes are relatively short, so Arctic insects face heightened consequences of warming if they are mismatched to their host-plant's phenology or if they are in the incorrect developmental stage at the onset of winter. If insect phenology shifts, multiple trophic levels can be impacted due to insects' critical roles as pollinators and food resources. Here I present the first analysis of phenological shifts in Alaskan butterflies in the context of a warming climate. I examined species records from Fairbanks, Alaska spanning five decades (1966-2016) for 15 butterfly species common in Interior Alaska during spring (April – June). Linear regressions were fit to test whether collection records within these months have advanced since 1966. Thirteen of 15 species advanced their spring flights, with advancement ranging from one to almost five days per decade. This pattern was consistent across phylogenetic groups and between taxa with differing life-history strategies, suggesting the effect is general across most butterfly species and likely other insect groups in Alaska.

Introduction

Phenology, or life-history timing, is the synchronization of a species' life history events within the annual cycle of climatic seasonality. In today's warming world, the phenologies of some species are shifting earlier in the calendar year, and this trend is especially pronounced in the Arctic (Valtonen *et al.* 2014, Høye *et al.* 2013, Høye *et al.* 2014, Post *et al.* 2018). The Arctic is highly seasonal, so migratory birds (and their insect prey) must time growth and reproduction to occur during the brief spring and summer before winter arrives (Rich *et al.* 2013, McDermott 2017). The timing of seasonal processes, including hydrological and vegetation, could have major impacts on higher trophic levels. A change in the timing of vegetation emergence, such as earlier green-up, could impact phytophagous and pollinating insects. Some arthropods are capable of matching their phenologies to the changing phenology of their host plants (Bartomeus *et al.* 2011, Iler *et al.* 2013, Bodis & Sramko 2015). However, if an asynchrony develops, plants and their arthropod associates are at risk of mismatch (Kudo & Ida, 2013, Ovaskainen *et al.* 2013, Visser & Both 2005). Phenologies are changing due to earlier spring warming, which has affected abiotic processes in Alaska such as the date of winter river ice break up. Spring break up on the Yukon, Tanana, and Kuskokwim Rivers has advanced an average of 1.3 days per decade since 1949 (Bieniek *et al.* 2011) as climates have warmed.

Earlier, warmer spring temperatures in Alaska have also been linked to the earlier bud break of leaves ("green-up"). Between 1975 and 2005, green-up appears to have advanced 3.3 days per decade in the tundra regions of Alaska's North Slope (Delbart & Picard, 2007). In Fairbanks, green-up has been measured from 1976 onward. Some of the earliest years of green-up have occurred in the last decade (Euskirchen *et al.* 2017). Hydrological and vegetation shifts

in seasonal timing in the Arctic are certain to impact animals which are reliant upon them (Chapin *et al.* 2012).

Phenological shifts can have major consequences for species reliant upon them, such as migratory bird species that rely upon an abundance of insects to feed their young (Thackeray *et al.* 2010). Numerous migratory birds use Alaska as a summer breeding ground, and some Arctic bird species were found to have advanced their clutch-initiation dates between 4 to 8 days per decade between 2002 – 2011 (Liebezeit *et al.* 2014).

Insects have experienced widespread recent declines attributed, in part, to climate change (Potts *et al.* 2010), and the phenology of species living at high latitudes may make them be especially susceptible to change (Høye *et al.* 2013, Loboda *et al.* 2018). At Zackenberg Research Station (74°28' N, 20°34' W) in Greenland, the butterfly *Boloria chariclea* was found to fly 1.2 days earlier per decade since 1996 (Høye *et al.* 2014). However, Høye *et al.*'s other focal species, *Colias hecla*, did not show a significant advancement over the same time period. Short-lived organisms like insects may respond rapidly to warming through a change in the timing of their life-cycles via accelerated development (Both *et al.* 2009a). This trend has been well documented in butterflies, where advancement in seasonal flight timing has been found in butterfly species inhabiting mid-latitude Canada (Westwood & Blair, 2010), the United States (Polgar *et al.* 2013) and Western Europe (Altermatt, 2012, Diamond *et al.* 2011). Insects that overwinter in immature stages (egg, larvae, pupae) use spring temperatures to trigger growth and fuel development of immature stages into sexually mature adults. In cold, high-latitude climates, spring warming is particularly important to the timing of this development in an often extremely contracted growing season (Danks, 1987).

Not all lepidopteran species changed their phenologies in the same way to changing climate at high latitudes. In Sweden, some species of butterflies have advanced their flights 3.8 days for every 1° C rise in temperature, although other species were found to emerge later in the season than historically (Karlsson 2014). In a study of moths in Finland (Valtonen *et al.* 2011), some species advanced their flight periods due to temperature increases, but photoperiod constrained the phenology of other species, indicating that not all Lepidoptera will respond uniformly to a temperature increase. Finnish noctuid moths are predicted to advance their flight season by 4.3 – 5.6 days per 1° C increase in temperature, which amounts to an advancement of 13-17 days per 3° C, a rate of warming which the Intergovernmental Panel on Climate Change predicts could be reached by 2090-2099 (Valtonen *et al.* 2014). Lepidoptera can provide useful “canaries-in-the-coalmine” to detect early signs of widespread phenological shifts in the overall insect fauna within an ecosystem.

Analysis of phenological trends requires insect records (either specimens or observations) that have been consistently collected over time. Butterflies are typically well documented across space and time compared to smaller, more cryptic, and less charismatic insects, which also are usually more difficult to identify. While the phenology of high-latitude butterflies is known to be advancing elsewhere, this paper summarizes the first analysis of Interior Alaska butterflies and their phenological patterns over the last half century. Here, I present an analysis to test for changes in phenology across 15 butterfly species found in Interior Alaska, including species from all five families of butterfly found in the state.

Methods

Study species and data sources. Seasonal records of butterfly species occurrences are available from 1966 onward from the area around Fairbanks, Alaska (64.8° N, 147.7° W). The vast majority of records were amassed by the late Dr. Kenelm W. Philip's Alaska Lepidoptera Survey. His survey was based in Fairbanks, and consequently, the Interior regions of Alaska immediately proximate to Fairbanks were the most consistently sampled. I therefore focused this phenological study on species records, both observations and specimens, collected in and around Fairbanks (Fig. 1). Dr. Philip's collecting trips outside of the Fairbanks area usually occurred in mid- to late June through July, and the Fairbanks butterfly community was sampled less consistently after the month of June. Thus, I limit my analysis to 15 common species (Table 1) observed from April to June (Philip & Ferris, 2016), collected or observed in and around Fairbanks between 1966 and 2005.

My analysis included records from both the Alaska Lepidoptera Survey along with additional specimen and observational butterfly records that were publicly available for the Fairbanks area. Butterfly records from the Alaska Lepidoptera Survey are part of the larger Kenelm W. Philip (KWP) collection currently held at the University of Alaska Museum (UAM). Records from both the KWP and UAM insect collections are publicly available through Arctos, a collections management platform (<http://arctos.database.museum>). Data from Arctos are included in the Global Biodiversity Information Facility (<https://www.gbif.org>), which aggregates both specimen and observational data for organisms, worldwide (GBIF Occurrence Downloads, 2017). Due to a formal agreement between Dr. Philip and the Smithsonian Institution (SI), eventually 90% of the pinned KWP collection specimens (and the associated data) will be transferred to the SI and the data will not be accessible in Arctos. Observational

databases which held Fairbanks butterfly records included both eButterfly (<http://www.e-butterfly.org>) and iNaturalist (<https://www.inaturalist.org>). Butterfly data for Fairbanks, Alaska (Fig 1) were downloaded from GBIF and were derived from the institutions listed in Table 1. A small number of additional species records were transcribed directly from specimen labels at the McGuire Center for Lepidoptera and Biodiversity (MGCL) in Gainesville, Florida, as the MGCL data were not available via GBIF. The records I used were grouped to species based on the taxonomic classification of Pohl *et al.* (2018).

Specimen identification. Specimens were identified by a variety of experts, students, and unknown contributors; specimen records from the KWP collection were all identified to species by Dr. Philip. Specimens from the University of Alaska Museum Insect Collection (UAM), Utah Museum of Natural History (UMNH), and Oklahoma Museum of Natural History (OMNH) were identified by a mixture of both known experts, students, and unknown contributors. I have reviewed the butterfly collection from UAM to ensure species used in this study were accurately identified using the field guide of Philip and Ferris (2016). Records from UMNH and OMNH were all of large and relatively distinct species (*Papilio machaon*, *Pontia occidentalis*, *Erebia epipsodea*) that are easily identifiable. Observational records submitted to iNaturalist and eButterfly are vetted by multiple experts. Alaskan material held at the McGuire Center was identified by Andy D. Warren, Jack L. Harry, or Floyd and June Preston.

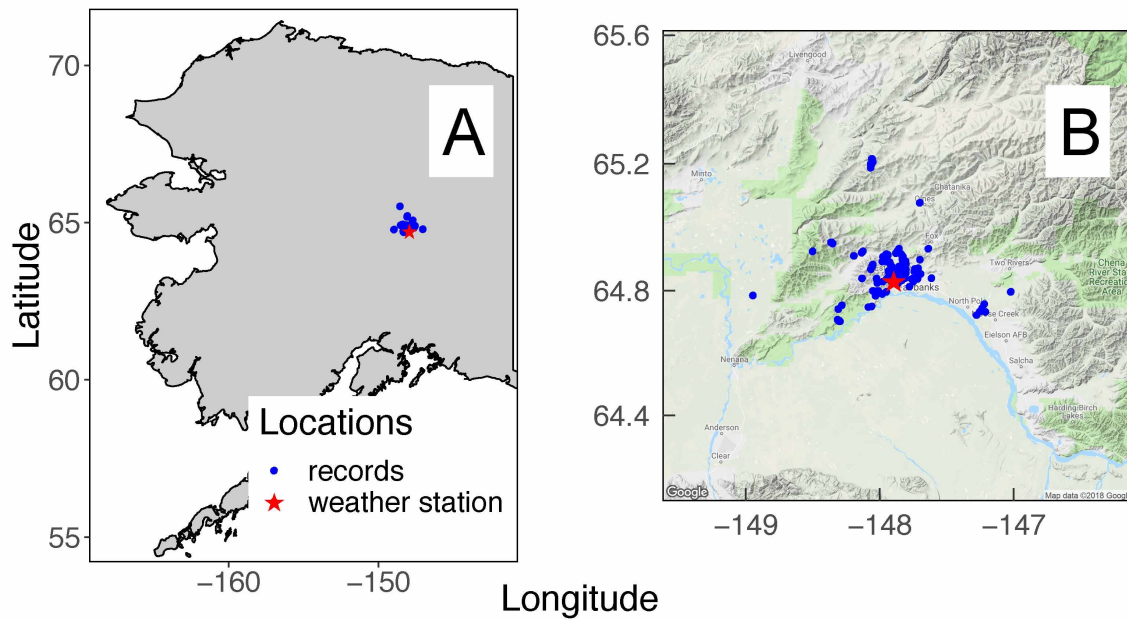


Figure 1. Location of the weather station at Fairbanks International Airport (red star) and the butterfly records (blue dots) for Fairbanks, Alaska derived from the Global Biodiversity Information Facility (GBIF) for *Papilio canadensis*, *Papilio machaon*, *Erynnis persius*, *Carterocephalus palaemon*, *Glaucopsyche lygdamus*, *Celastrina lucia*, *Callophrys polios*, *Callophrys augustinus*, *Colias canadensis*, *Pontia occidentalis*, *Boloria frigga*, *Boloria freija*, *Erebia discoidalis*, *Erebia epipsodea*, and *Erebia mancinus* documented from 1966 onward. Study area within Alaska (Panel A) near Fairbanks (Panel B). Plot made using ggplot2 (Wickham, 2016).

Defining Phenological Shift. There is no general consensus about how to measure and evaluate phenological shifts in butterflies. Previous phenological studies of butterflies have used the first-sighting date (Forister & Shapiro 2003), or peak-appearance date (Roy & Sparks 2000) alone or in combination with the mean seasonal flight date (Stefanescu *et al.* 2003). Van Strien *et al.* (2008) suggested that using only first-flight dates to gauge phenological changes can be biased if either the number of sampled locations or a population itself increases over time, since a larger population would have more phenological outliers available to be detected, but this bias would be limited if a fixed percentage of records each year are analyzed. For example, using only a percentage of butterfly records, such as the first 20% of all records per species for each year (*e.g.* Polgar *et al.* 2013) can explain more variation in flight period trends than using records from the entire season.

Phenology analyses of museum specimen records for butterflies have also used the mean first-flight date at localities of interest to account for unequal sampling effort across time and space (Kharouba *et al.* 2014). Annual spring collecting efforts of the Alaska Lepidoptera Survey occurred at several localities near Fairbanks, and thus maintained relatively consistent geographic localities throughout the time series. However, survey record keeping varied through time; it ran through 2013 but digital records stopped in 2005. Digitization of specimen records is now ongoing, but many species have only sparse specimen records after 2005. This required some accommodation in the statistical analyses, which I detail below in the “Data Analysis” section.

Temperature Data. Daily minimum and maximum temperature data for the Fairbanks International Airport (64.81° N, 147.86° W) were obtained through the National Center for

Environmental Information (<https://www.ncdc.noaa.gov/>). These values were used to calculate a daily mean temperature. Mean daily temperature was used to calculate a conservative measurement of accumulated growing degree days (GDD), or total thermal energy gain during the growing season (Parry & Carter 1985, Gordon & Bootsma, 1993) with a base of 5°C. Cumulative growing degree days were calculated up to the date that each specimen was collected, so that the amount of heat experienced by each individual could be used as an explanatory variable in subsequent analyses to understand if increasing heat availability explained earlier observation or collection dates.

Statistical analysis. Analyses were conducted in R, version 3.4.2 (R Core Team, 2018), using the packages “olsrr” (Hebbali, 2017) and “lava.” To test whether butterflies are flying earlier in spring over time, I regressed calendar date (day of year) and year for all available species records for the months of April, May, and June for Fairbanks, Alaska. My null hypothesis was that the slope of the linear regression was zero; in other words, that butterflies were flying at the same time across the sampling period beginning in 1966. Standardized (studentized) residuals were examined for all species to test for outliers. Leverage (a test of whether any individual datum significantly influences the linear regression) was calculated for each year for each species across the entire range of years (1966-2017). If leverage appeared higher than 3% for records post-2005 when the digitization data ended, a new linear model was calculated using only data up to either 2005 or to the year when leverage began to rise. Of all of the study species, *Carterocephalus palaemon* was the least well-recorded in recent years and exhibited high leverage for all records post-1990; so only 24 years of data (1966-1990) were analyzed (Table 1).

I also hypothesized that each butterfly species was flying at the same thermal date over time – that they would emerge to fly after a given accumulation of GDD rather than on a specific calendar date. I associated all butterfly records with GDD data for their collection date to represent heat availability, and tested this hypothesis by performing linear regressions for each species, with the GDD value as the dependent variable and year as the independent variable. If true, this relationship should have a slope of zero and indicate that emergence date is entirely a function of accumulated GDD rather than corresponding to a specific calendar date or being caused by another seasonal cue like day length.

Results

Cumulative annual GDD in Fairbanks, Alaska (Fig.2, left) has increased $42.8 \text{ GDD} \pm 6.2 \text{ SE}$ in total seasonal heat per decade since 1930, the year that temperature records began. Butterfly specimens were collected from 1966 onward, and between 1966 and 2016, seasonal warming in Fairbanks measured as cumulative GDD through June 30 (DOY = 181) has increased $18.7 \text{ GDD} \pm 0.81 \text{ SE}$ (Fig. 2, right).

Using temperature and GDD data for the day and year of collection records, I found that butterfly flight periods have occurred at the same *temperatures* over time for each species. Butterflies are emerging after accumulating the same amount of heat over time; there was no significant difference ($p > 0.05$) in flight period temperature over time for any species. It follows that their critical emergence temperatures are occurring earlier in the season due to warmer spring temperatures. The majority of Fairbanks butterfly species (13 of 15) show significant advancement ($p < 0.05$) in the date of their first flights over the last 51 years of records (1966-2017). However, the trend is not universal across species or within butterfly families (Table 1).

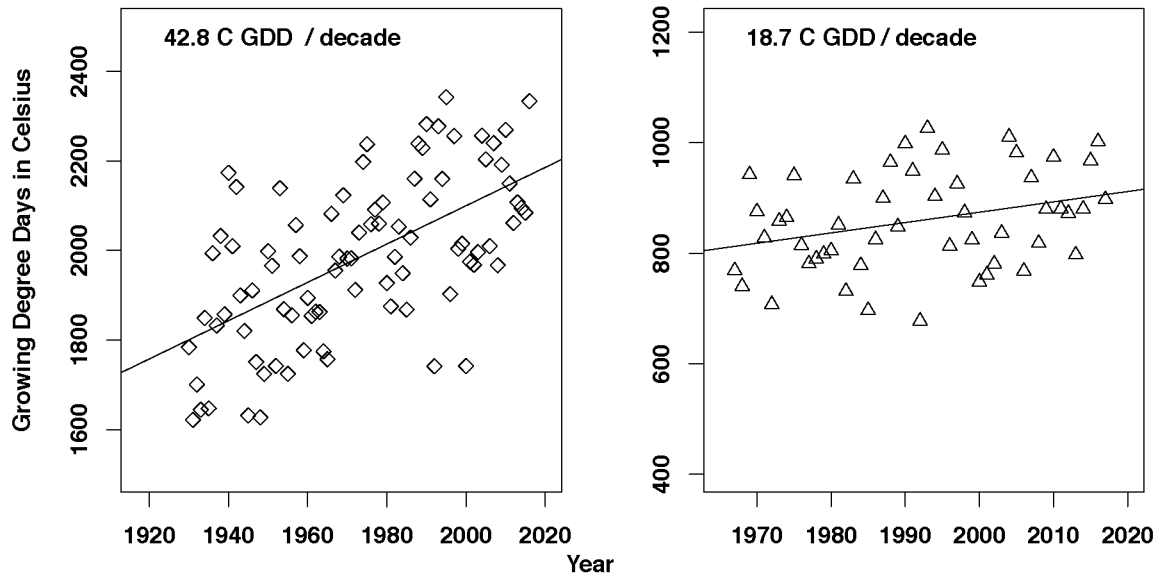


Figure 2. Annual accumulated growing degree days (GDD) calculated from a base of 0°C for Fairbanks, Alaska from 1930 to 2016 (left). Since 1930 GDD have increased by $42.8 \text{ GDD} \pm 6.2 \text{ SE}$ per decade (adjusted R-squared = 0.3473, $F = 46.76$, $p < 0.001$). Between 1966 and 2016, cumulative growing degree days $^{\circ}\text{C}$ as of 30 June of each year increased $18.7 \pm 0.81 \text{ SE}$ per decade (Adjusted R-squared = 0.07878, $p = 0.0259$).

Table 1: Species records for Fairbanks, specimen records obtained from collections including (1) Kenelm W. Philip Lepidoptera Collection (KWP), (2) University of Alaska Museum (UAM), (3) Utah Museum of Natural History (UMNH), (4) Oklahoma Museum of Natural History (OMNH) and the (5) McGuire Center for Lepidoptera and Biodiversity (MGCL). Observational records obtained from (6) iNaturalist / eButterfly. Significant advancement trends (days per year) : p value <0.05 (*), p <0.01 (**), p<0.001 (***)

Family	Species	Total Records	Days / year trend	Span of Records	Sources
Papilionidae	<i>Papilio canadensis</i>	203	-0.389***	39 years	1,2,3,6
Papilionidae	<i>Papilio machaon</i>	162	-0.229**	39 years	1,2
Hesperiidae	<i>Erynnis persius</i>	140	-0.387**	29 years	1,2
Hesperiidae	<i>Carterocephalus palaemon</i>	53	-0.487**	24 years	1,2,5
Lycaenidae	<i>Glaucopsyche lygdamus</i>	346	-0.143**	51 years	1,2
Lycaenidae	<i>Celastrina lucia</i>	239	-0.021	51 years	1,2,3
Lycaenidae	<i>Callophrys polios</i>	392	-0.099 *	39 years	1,2
Lycaenidae	<i>Callophrys augustinus</i>	304	-0.198***	39 years	1,2
Pieridae	<i>Colias canadensis</i>	516	-0.475***	35 years	1,2
Pieridae	<i>Pontia occidentalis</i>	38	+0.216	51 years	1,2,6
Nymphalidae	<i>Boloria frigga</i>	293	-0.131***	51 years	1,2
Nymphalidae	<i>Boloria freija</i>	405	-0.181***	39 years	1,2,4
Nymphalidae	<i>Erebia discoidalis</i>	261	-0.244***	51 years	1,2,3
Nymphalidae	<i>Erebia epipsodea</i>	104	-0.149***	51 years	1,2,4,6
Nymphalidae	<i>Erebia mancinus</i>	182	-0.255***	51 years	1,2

Both species of swallowtail butterflies (Papilionidae: *Papilio*) in Fairbanks exhibited significant seasonal advancement. The timing of the first flights of *P. canadensis* (Tiger Swallowtail) advanced by 2.8 ± 0.5 SE days per decade ($R^2 = 0.1406$; $F = 30.93$; $p < 0.001$) over the last 51 years. Leverage ranged from 3% to 7% in observations of *P. canadensis* made after 2005 (Fig. 3, top left). Points drawing leverage higher than 3% were removed and a subsequent trend analyses was conducted using 39 years of records from 1966-2005 (Fig. 3, bottom left). The advancement trend became slightly stronger for *P. canadensis*, with an advancement of 3.9 ± 0.5 SE days per decade ($R^2 = 0.216$, $F = 49.78$, $p < 0.001$) over this 39 year time period. The interpretation of the results is effectively identical regardless of whether highly leveraged points are removed or not.

Similarly, *P. machaon* (Old World Swallowtail) exhibited an advancement of 2.2 ± 0.6 SE days per decade ($R^2 = 0.0582$, $F = 10.95$, $p < 0.001$) over 51 years. Leverage of records ranged from 3% - 10% for observations made after 2005. In a subsequent analysis of records only from 1966-2005, *P. machaon* showed an advancement of 2.3 ± 0.8 SE days per decade ($R^2 = 0.03773$, $F = 7.077$, $p < 0.001$) (Fig. 3, top right).

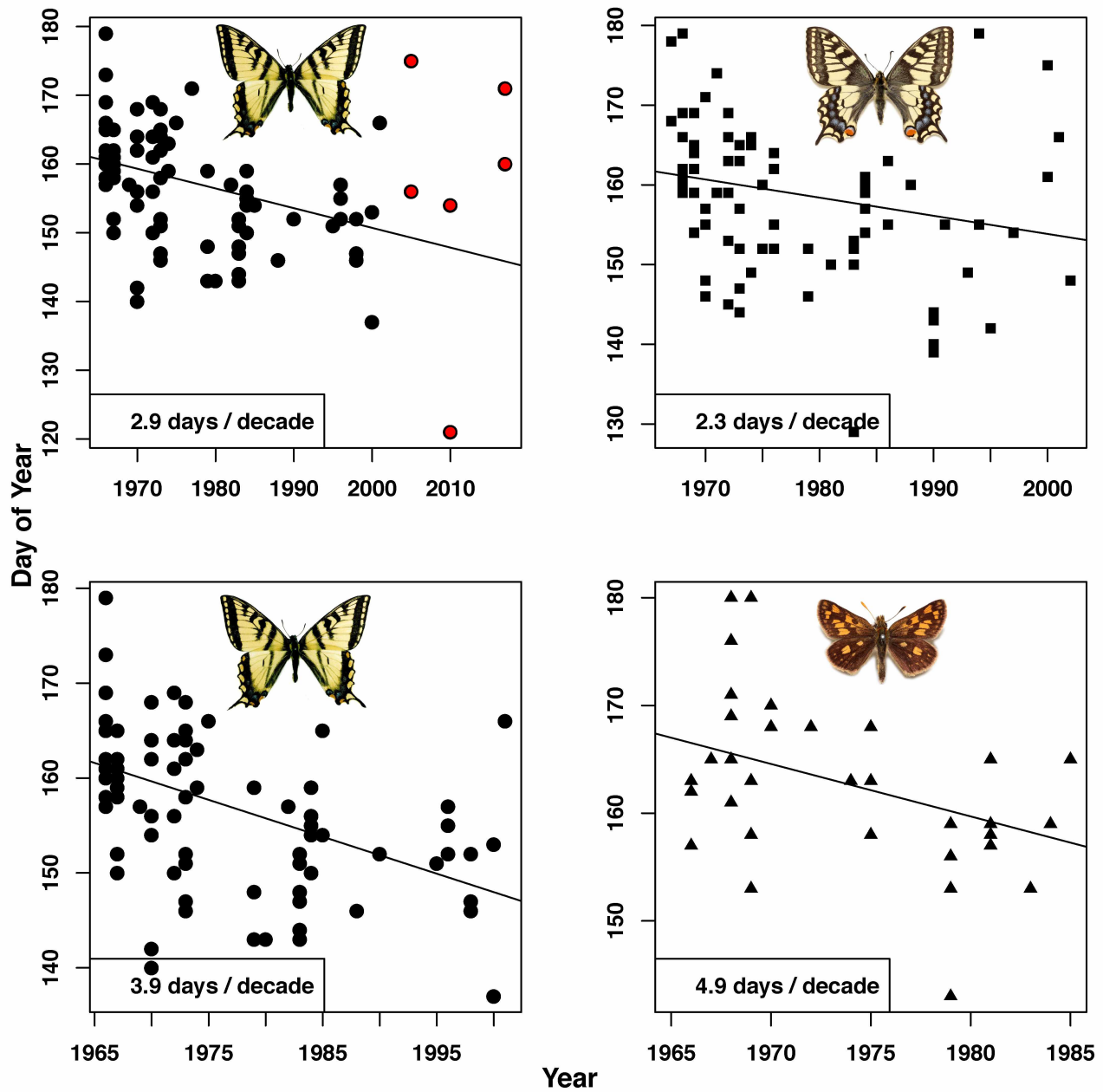


Figure 3. Spring emergence trends for species in Fairbanks: *Papilio canadensis* (left column) records (top left) depicting all records which caused significant influence on the linear regression prediction; leverage values larger than 3% are shown in red. *P. canadensis* records from 1966-2005 (bottom left) were subsequently analyzed. *Papilio machaon* records (top right) were analyzed from 1966-2005 and *Carterocephalus palaemon* (bottom right) were analyzed from 1966-1990. Butterfly images used with permission of Warren *et al.* (2013).

Of the four species of skipper butterflies (Hesperiidae) found in Alaska, only two, *Carterocephalus palaemon* (Pallas 1771) and *Erynnis persius* (Scudder 1863), are spring fliers. Both species have been poorly collected over time, which is not unusual for small, inconspicuous taxa, and they are not well-represented in recent decades. Nonetheless, *C. palaemon* exhibited an advancement of 2.8 ± 0.8 SE days over the full 51-year dataset ($R^2 = 0.1755$, $F = 10.86$, $p < 0.001$). However, it was poorly collected after 1990, and so the extrapolation for the last 27 years was tested for leverage. Leverage calculated for records of *C. palaemon* using the full dataset ranged from 3% - 30% with the highest values for observations made after 1990. Subsequent analysis of the shortened 24-year dataset (1966-1990) showed an advancement of 4.8 ± 1.5 SE days per decade ($R^2 = 0.1723$, $F = 9.786$, $p = 0.003017$) (Fig. 3, bottom right). *Erynnis persius* records ended in 1990 for Fairbanks, but analysis of 24 years of data (Table 1) showed a significant measurable advancement of 3.8 ± 1.4 SE days per decade ($R^2 = 0.05146$, $F = 7.052$, $p = 0.008906$). Regression plots for *Erynnis persius* and the remainder of species analyzed are available in Appendix A.

Two early-flying, white and sulfur butterflies (Pieridae) were included in this analysis. *Colias canadensis* Ferris 1982 had records between 1970 and 2005 (Table 1) and exhibited a strong advancement of 4.7 ± 0.6 SE days ($R^2 = 0.1002$, $F = 56.82$, $p < 0.001$). *Pontia occidentalis* (Reakirt 1866) showed a non-significant delay in flight period, (Table 1) meaning it is flying 2.2 ± 1.4 SE days later per decade ($R^2 = 0.05944$, $F = 2.275$, $p = 0.1402$) over 51 years. However, this is likely due to a proliferation of a second seasonal generation flying earlier in later years, which, given the way the analysis was structured, obscured estimates of the first flight date (see Discussion section).

Among the four lycaenid species analyzed (Table 1), *Celastrina lucia* (Kirby 1837) had no significant change in flight period over 51 years ($p = 0.667$) while *Glaucopsyche lygdamus* (Doubleday 1841) advanced 1.4 ± 0.4 SE days ($R^2 = 0.03035$, $F = 10.77$, $p = 0.001138$) over the same time period. *Callophrys polios* (Cook and Watson 1907) records from after 2005 had a leverage of almost 2.5% compared to all other records. Subsequent analysis of data for this species from 1966 to 2005 revealed advancement of 0.9 ± 0.4 SE days ($R^2 = 0.01369$, $F = 5.4$, $p = 0.02065$) over 39 years. *Callophrys augustinus* (Westwood 1852) observations were also restricted to 1966-2005 and had a somewhat stronger progression of the flight period of 2.0 ± 0.4 SE days per decade ($R^2 = 0.06262$, $F = 20.17$, $p < 0.001$).

The majority of spring-flying nymphalid butterflies in Fairbanks are well-collected, making it possible to analyze species from the genera *Boloria* and *Erebia* (Table 1). *Boloria frigga* (Thunberg 1791) advanced 1.3 ± 0.3 SE days per decade ($R^2 = 0.06089$, $F = 18.28$, $p < 0.001$) over the 51-year period. *Boloria freija* (Thunberg 1791) records were sporadic after 2005, but from 1966 – 2005 the species had an advancement of 1.8 ± 0.4 SE days per decade ($R^2 = 0.0485$, $F = 19.83$, $p < 0.001$). Over the 51-year period, *Erebia discoidalis* (Kirby 1837) advanced 2.1 ± 0.3 SE days per decade ($R^2 = 0.1356$, $F = 37.95$, $p < 0.001$). *Erebia epipsodea* Butler 1868 was not collected between 1980 and 2000; nonetheless a linear regression of available records (over the 51-year time period) showed advancement of 1.5 ± 0.2 SE days per decade ($R^2 = 0.2848$, $F = 40.62$, $p < 0.001$). *Erebia mancinus* (Doubleday 1849) advanced 2.5 ± 0.3 SE days per decade ($R^2 = 0.2218$, $F = 51.31$, $p < 0.001$) between 1966 and 2017.

Discussion

Flight periods of the majority of spring-flying Fairbanks butterfly species have advanced significantly, but the trend differs between species. The papilionids, *P. canadensis* and *P. machaon*, both exhibited significantly earlier flights, but *P. canadensis* exhibited a stronger seasonal response of almost four days per decade compared to two days per decade for *P. machaon*. *Papilio canadensis* breeds and flies in lowland boreal forest and its larvae feed on a variety of deciduous trees including willow and aspen (Philip & Ferris, 2016). *Papilio machaon* generally is associated with higher-elevation ridges above treeline where its larvae feed on *Artemisia*. It is possible that warming in the boreal lowland forests around Fairbanks has occurred at a faster rate than the higher elevation ridges where *P. machaon* is found.

The skipper butterflies, *Carterocephalus palaemon* and *Erynnis persius*, (Hesperiidae) also show advancement over time, but lacked records for recent decades. These species are poorly collected in Fairbanks due to their small size and fast flight, so phenology trends remain understudied for the hesperiid family.

Of the pierid butterflies analyzed, *Colias canadensis* advanced its flight timing, whereas unlike all other species, *Pontia occidentalis* showed a detectably later seasonal flight period over time. *Pontia occidentalis* is one of the few multivoltine species in Fairbanks, and it is likely that records spanning April – June include the second brood of the season, especially later in the time series as flights advanced earlier in the season. This species has been reared in laboratory conditions and exhibits seasonal polyphenism in Fairbanks (Shapiro, 1975), and so discerning between broods would require a large sample of specimens within each year. The non-significant effect I found is likely due to the second generation emerging earlier in the year and overlapping in time with the first generation. Prior to ca. 1990, the second generation probably did not

emerge until after July 1, outside the window of my spring analyses, while in later years, the second generation likely began emerging earlier in June, inside the temporal window of the analysis.

Of the four lycaenid species I analyzed, the two “blues,” *Celastrina lucia* and *Glaucopsyche lygdamus*, showed divergent responses, with only *G. lygdamus* exhibiting an advancement in flight period. It is notable that this species is found in both boreal forest and tundra habitats, whereas *C. lucia* occurs only in the boreal forest. Both these species probably feed on legumes, but the preferred host plant species are unknown (Philip & Ferris, 2016). Differences in the timing of host plant phenology may be causing these species’ divergent responses.

Both of the “elfins”, *Callophrys polios* and *Callophrys augustinus*, displayed significant trends, but despite identical flight periods the magnitude of the trend was almost double in *C. augustinus*. Each is thought to share the same habitat of boreal forest and both are thought to feed on *Arctostaphylos uva-ursi* (Ericaceae), but *C. polios* flies earlier than *C. augustinus*, with *C. polios* records beginning on mean day of year 140 (supplemental Fig. 6) or May 21st, compared to day 150 (supplemental Fig. 7). Therefore, *C. augustinus* experiences more spring heat prior to adult emergence than *C. polios* in a given season. Warming may be unequal across the spring season, with most warming occurring in May.

All the nymphalids revealed a significant and similar shift in the timing of first-flight between 1.3 – 2.5 days earlier per decade. Grass-feeding species studied within the genus *Erebia* had the strongest response of all the nymphalids, flying 2.4 to 2.5 days earlier every decade since 1966.

The phenological trends displayed by butterflies of all five families found in Alaska reveal shifts to earlier seasonal flights. The pattern of advancing phenology demonstrated in butterflies is likely to be shared by other insect groups similarly responding to warming temperatures. A major shift in insect emergence timing could lead to consequences both within and between trophic levels. For insects, emergence that occurs too early risks places species in the wrong life stage to survive the subsequent winter – a concept described in the “lost-generation hypothesis” (Van Dyck *et al.* 2015). This is an important consideration for conservation predictions of high-latitude species, and phenology analyses allow for the first indication of highly temperature sensitive species. Climatic warming has been found to increase voltinism in European butterflies (Altermatt, 2010) although it is unknown what threshold of temperature increase would cause this response in Subarctic Alaskan populations such as those in the Fairbanks area.

Insects are a crucial food source for migratory birds to Alaska (Rich *et al.* 2013) so shifts to earlier seasonal flight could have large-scale consequences for avian species that are unable to time their arrival and reproduction to accommodate the earlier seasonal abundance of food. This could lead to a trophic-level mismatch between birds and insects (Both *et al.* 2009b, Parmesan & Yohe 2003). Shifting insect phenology could also impact lower trophic levels if herbivorous insects emerge before their host-plants have matured for consumption or pollination (Singer & Parmesan, 2010). Climatological mismatches between trophic levels have been reported from both terrestrial and aquatic systems in multiple biomes (Stenseth *et al.* 2002, Parmesan & Yohe, 2003). Butterflies, as “canaries-in-the-coalmine”, have revealed signs of widespread phenological shifts which may be found across the insect fauna within the boreal forests of Fairbanks and greater Alaska.

Temperatures continue to rise in Alaska, and the phenology of many insects is expected to advance commensurately, but the effects are undoubtedly of much broader significance. Winter and spring warming trends in northwestern Canada have shown even greater warming than Alaska, increasing between 2.6 and 3°C since 1948 (Lemmen *et al.* 2008). Annual temperatures in Interior Alaska (Fairbanks), by comparison, have increased only 1.4 °C since 1948, with the strongest seasonal warming occurring in winter months of December and January (2.2-2.5°C) and April (3.1°C) (Wendler & Shulski 2009). In Alaska, temperatures have increased the most from 1976 onward, after a sudden decrease in Pacific oceanic surface pressure resulted in an increase in the advection of relatively warm air into Alaska caused by shift to the positive phase of the Pacific Decadal Oscillation (PDO) (Mantua & Hare 2002). This shift caused increases in mean annual temperature, cloudiness, precipitation, and windspeed from 1977 onward compared to the preceding 25 years (1951 – 1975) in Fairbanks (Hartmann & Wendler, 2005). As temperatures continue to warm and advance the growing season, insects will respond through earlier emergence, but the impact on other trophic levels remains unknown.

Literature Cited

- Altermatt, F. (2010). Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society of London: Biological Sciences*, 277(1685): 1281-1287.
- Altermatt, F. (2012). Temperature-related shifts in butterfly phenology depend on the habitat. *Global Change Biology*, 18(8): 2429-2438.
- Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., Colla, S., Kornbluth, S., & Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences*, 108(51): 20645-20649.
- Bieniek, P. A., Bhatt, U. S., Rundquist, L. A., Lindsey, S. D., Zhang, X., & Thoman, R. L. (2011). Large-scale climate controls of interior Alaska river ice breakup. *Journal of Climate*, 24(1): 286-297.
- Bodis, J., & Sramko, G. (2015). No evidence for historical declines in pollination success in Hungarian orchids. *Applied Ecology and Environmental Research*, 13(4): 1097-1108.
- Both, C., Van Asch, M., Bijlsma, R. G., Van Den Burg, A. B., & Visser, M. E. (2009). Climate change and unequal phenological changes across four trophic levels: constraints or adaptations? *Journal of Animal Ecology*, 78(1): 73-83.
- Both, C., Van Turnhout, C. A., Bijlsma, R. G., Siepel, H., Van Strien, A. J., & Foppen, R. P. (2009). Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. *Proceedings of the Royal Society of London B: Biological Sciences*, 15(25): 1-8.

- Breed, G. A., Stichter, S., & Crone, E. E. (2013). Climate-driven changes in northeastern US butterfly communities. *Nature Climate Change*, 3(2): 142-145
- Chapin III, F. S., Jefferies, R. L., Reynolds, J. F., Shaver, G. R., Svoboda, J., & Chu, E. W. (Eds.). (2012). Arctic Ecosystems in a Changing Climate: An Ecophysiological Perspective. *Academic Press*. (p.469)
- Danks, H. V. (1987). Insect dormancy: an ecological perspective. *Biological Survey of Canada (Terrestrial Arthropods)*. (p.439)
- Delbart, N., & Picard, G. (2007). Modeling the date of leaf appearance in low-arctic tundra. *Global Change Biology*, 13(12): 2551-2562.
- Diamond, S. E., Frame, A. M., Martin, R. A., & Buckley, L. B. (2011). Species' traits predict phenological responses to climate change in butterflies. *Ecology*, 92(5):1005-1012.
- Euskirchen, E. (2017). Greenup values for interior Alaska 1976–Present, Bonanza Creek LTER–University of Alaska Fairbanks, BNZ: 300. (data from: Anderson J., C. Elsner, T. Fathauer, E. S. Euskirchen. 2017. Greenup values for interior alaska 1976 – Present) *Environmental Data Initiative*. <http://dx.doi.org/10.6073/pasta/08050a3f7b1fbc5ae7cac378feaaa6f1>. Dataset accessed 12/15/2017.
- Forister, M. L., & Shapiro, A. M. (2003). Climatic trends and advancing spring flight of butterflies in lowland California. *Global Change Biology*, 9(7): 1130-1135.
- [GBIF.org](https://doi.org/10.15468/dl.m5keku) (2nd December 2017) GBIF Occurrence Download <https://doi.org/10.15468/dl.m5keku> (*Boloria chariclea*)
- [GBIF.org](https://doi.org/10.15468/dl.cknnzt) (28th November 2017) GBIF Occurrence Download <https://doi.org/10.15468/dl.cknnzt> (*Oeneis bore*, *Oeneis polixenes*, *Erebia discoidalis*, *Erebia mancinus*, *Erebia epipsodea*, *Polygonia faunus*, *Nymphalis antiopa*, *Nymphalis l-album*, *Aglais milberti*, *Boloria polaris*, *Boloria frigga*, *Boloria freija*, *Pontia occidentalis*, *Euchloe creusa*, *Euchloe ausonides*)

[GBIF.org](#) (28th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.vxkiss> (*Callophrys augustinus*)

[GBIF.org](#) (28th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.nujccf> (*Glaucopsyche lygdamus* & *Celastrina lucia*)

[GBIF.org](#) (28th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.q17zos> (*Callophrys polios*)

[GBIF.org](#) (27th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.rug7cn> (*Colias candensis*)

[GBIF.org](#) (27th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.xxwz1p> (*Carterocephalus palaemon*)

[GBIF.org](#) (27th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.8alfhf> (*Erynnis persius*)

[GBIF.org](#) (27th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.obuy1b> (*Papilio machaon*)

[GBIF.org](#) (27th November 2017) GBIF Occurrence
Download <https://doi.org/10.15468/dl.khl7fu> (*Papilio canadensis*)

Gordon, R., & Bootsma, A. (1993). Analyses of growing degree-days for agriculture in Atlantic Canada. *Climate Research*, 169-176.

Hartmann, B., & Wendler, G. (2005). The significance of the 1976 Pacific climate shift in the climatology of Alaska. *Journal of Climate*, 18(22): 4824-4839.

Hebbali, A. (2017). olsrr: Tools for Building OLS Regression Models. R package version 0.4.0
<https://CRAN.R-project.org/package=olsrr>

- Høye, T. T., Post, E., Schmidt, N. M., Trøjelsgaard, K., & Forchhammer, M. C. (2013). Shorter flowering seasons and declining abundance of flower visitors in a warmer Arctic. *Nature Climate Change*, 3(8): 759-763.
- Høye, T. T., Eskildsen, A., Hansen, R. R., Bowden, J. J., Schmidt, N. M., & Kissling, W. D. (2014). Phenology of high-arctic butterflies and their floral resources: species-specific responses to climate change. *Current Zoology*, 60(2): 243-251.
- Iler, A. M., Inouye, D. W., Høye, T. T., Miller-Rushing, A. J., Burkle, L. A., & Johnston, E. B. (2013). Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. *Global Change Biology*, 19(8): 2348-2359.
- Karlsson, B. (2014). Extended season for northern butterflies. *International Journal of Biometeorology*, 58(5): 691-701.
- Kharouba, H. M., Paquette, S. R., Kerr, J. T., & Vellend, M. (2014). Predicting the sensitivity of butterfly phenology to temperature over the past century. *Global Change Biology*, 20(2): 504-514.
- Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, 94(10): 2311-2320.
- Liebezeit, J. R., Gurney, K. E. B., Budde, M., Zack, S., & Ward, D. (2014). Phenological advancement in arctic bird species: relative importance of snow melt and ecological factors. *Polar Biology*, 37(9): 1309-1320.
- Lemmen, D. S., Warren, F. J., Lacroix, J., & Bush, E. (2008). From impacts to adaptation: Canada in a changing climate. *Government of Canada, Ottawa*.

- Loboda, S., Savage, J., Buddle, C. M., Schmidt, N. M., & Høye, T. T. (2018). Declining diversity and abundance of High Arctic fly assemblages over two decades of rapid climate warming. *Ecography*, 41(2): 265-277.
- Mantua, N. J., & Hare, S. R. (2002). The Pacific decadal oscillation. *Journal of Oceanography*, 58(1): 35-44.
- McDermott, M. T. (2017). Arthropod communities and passerine diet: effects of shrub expansion in Western Alaska. University of Alaska Fairbanks MS Thesis.
- Ovaskainen, O., Skorokhodova, S., Yakovleva, M., Sukhov, A., Kutenkov, A., Kutenkova, N., Shcherbakov, A., Meyke, E. and del Mar Delgado, M., (2013). Community-level phenological response to climate change. *Proceedings of the National Academy of Sciences*, 110(33): 13434-13439.
- Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421(6918): 37.
- Parry, M. L., & Carter, T. R. (1985). The effect of climatic variations on agricultural risk. *Climatic Change*, 7(1): 95-110.
- Philip, K.W., Ferris, C.D. (2016). Butterflies of Alaska: A Field Guide. 2nd ed. *Alaska Entomological Society*. (p. 110)
- Polgar, C. A., Primack, R. B., Williams, E. H., Stichter, S., & Hitchcock, C. (2013). Climate effects on the flight period of Lycaenid butterflies in Massachusetts. *Biological Conservation*, 160: 25-31.
- Post, E., Steinman, B. A., & Mann, M. E. (2018). Acceleration of phenological advance and warming with latitude over the past century. *Scientific Reports*, 8(1): 3927.

- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6): 345-353.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rich, M. E., Gough, L., & Boelman, N. T. (2013). Arctic arthropod assemblages in habitats of differing shrub dominance. *Ecography*, 36(9): 994-1003.
- Shapiro, A. M. (1975). Photoperiodic control of development and phenotype in a subarctic population of *Pieris occidentalis* (Lepidoptera: Pieridae). *The Canadian Entomologist*, 107(7): 775-779.
- Stenseth, N. C., Mysterud, A., Ottersen, G., Hurrell, J. W., Chan, K. S., & Lima, M. (2002). Ecological effects of climate fluctuations. *Science*, 297(5585): 1292-1296.
- Thackeray, S.J., Sparks, T.H., Frederiksen, M., Burthe, S., Bacon, P.J., Bell, J.R., Botham, M.S., Brereton, T.M., Bright, P.W., Carvalho, L. and Clutton-Brock, T.I.M. (2010). Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology*, 16(12), pp.3304-3313.
- Van Dyck, H., Bonte, D., Puls, R., Gotthard, K., & Maes, D. (2015). The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? *Oikos*, 124(1): 54-61.
- Van Strien, A. J., Plantenga, W. F., Soldaat, L. L., Van Swaay, C. A., & WallisDeVries, M. F. (2008). Bias in phenology assessments based on first appearance data of butterflies. *Oecologia*, 156(1): 227-235.

- Valtonen, A., Ayres, M. P., Roininen, H., Pöyry, J., & Leinonen, R. (2011). Environmental controls on the phenology of moths: predicting plasticity and constraint under climate change. *Oecologia*, 165(1): 237-248.
- Valtonen, A., Leinonen, R., Pöyry, J., Roininen, H., Tuomela, J., & Ayres, M. P. (2014). Is climate warming more consequential towards poles? The phenology of Lepidoptera in Finland. *Global Change Biology*, 20(1): 16-27.
- Visser, M. E., & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society of London: Biological Sciences*, 272(1581): 2561-2569.
- Warren, A. D., K. J. Davis, E. M. Stangeland, J. P. Pelham & N. V. Grishin. 2013.
[Illustrated Lists of American Butterflies.](#)
<http://www.butterfliesofamerica.com/>
- Wendler, G., & Shulski, M. (2009). A century of climate change for Fairbanks, Alaska. *Arctic*, 62(3): 295-300.
- Westwood, A. R., & Blair, D. (2010). Effect of regional climate warming on the phenology of butterflies in boreal forests in Manitoba, Canada. *Environmental Entomology*, 39(4): 1122-1133.
- Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

General Discussion

Alaskan biomes are shifting; increased productivity at the boreal forest – tundra ecotone has occurred in combination with a decline in primary productivity of interior spruce forests (Juday *et al.* 2015). At the southern edges of boreal forests, grassland has expanded into boreal forest (Beck *et al.* 2011) while shrubs are increasing in the tundra (Sturm *et al.* 2005). A lengthening growing season in Alaska is occurring in combination with earlier seasonal snowmelt (Stone *et al.* 2002). As the structure and timing of Alaskan vegetation are affected by climate warming, butterflies have also responded as expressed by changes in phenology and morphology.

Butterfly populations within Alaska have experienced decreased wing length and earlier flight periods as local habitats have experienced rising temperatures, corroborating previous reports of phenological and morphological differences across thermal scales. Phenological responses are known to be unequal across the latitudinal range of insects based on local climate conditions (Posledovich *et al.* 2014, Tauber *et al.* 1986). Morphological gradients in body size have also been reported across latitudinal and elevational clines for insects due to differences in temperature exposure (Alpatov 1929, Masaki 1978).

Environmentally-related body size shifts have been reported in insect taxa across deep geologic time; arthropod gigantism in the late Palaeozoic was proposed to be a combined result of a relatively hyperoxic and dense atmosphere (Harlé 1911), which promoted increased physiological development and aerodynamic resistance (Dudley 1998). Gigantism in insects has also been documented across diverse atmospheric and thermal conditions, so a stable ecological infrastructure including benefits such as abundant resources or low threats must also be important to body size increases (Vermeij 2016). A lack of specific predators could have driven

extreme arthropod body size through a lack of competition (Braddy *et al.* 2008) or through niche displacement, where large arthropods evolved to fill predatory trophic level roles that were later filled by vertebrates (Harrison *et al.* 2010). While there is certain evidence that the body sizes of some arthropods increased during these ‘optimal conditions,’ the vast majority remained small (Beckemeyer & Hall 2007) implying divergent responses to climatic change across taxa which could have been influenced by other factors such as species’ life history, along with a general ecological pattern that large animals, regardless of taxon, are generally rarer than smaller animals.

Intraspecific variation in response to climate warming has recently been reported (Tseng *et al.* 2018) in multiple species of ground beetles (Coleoptera: Carabidae) in British Columbia, Canada, where the largest four measured species decreased significantly in size compared to the smaller-bodied species measured. Tseng *et al.* (2018) reported varying levels of size-response among species to 26 measured climatic variables such as maximum autumn and spring temperature, precipitation, and heat moisture index, and proposed that the availability of required resources due to these changing variables among species may drive the difference in responses. In a rapidly changing world, life history strategy should be considered an important factor in determining extant species who may be sensitive to climate warming. However, the life-history strategies of many arthropods, including Alaskan butterflies, are not yet fully understood (Philip & Ferris 2016). While underlying mechanisms of species-level responses to warming may remain elusive, it is crucial to assess phenological and morphological responses to changing climatic conditions now that it is clear that Alaska’s insect fauna is changing rapidly in response to warming temperatures.

Natural history collections and their records can serve as fundamental sources of insight into patterns of changing morphology and timing of seasonal flight. Using museum collections to quantify arthropod responses due to anthropogenic warming can reveal extant species responses and allow for inferences of species conservation risk. Natural history collections are a priceless resource for comprehending the natural world, but their value is often underappreciated because of a lack of connection about their importance to society (Suarez *et al.* 2004). Insect collections which span both space and time are essential tools for quantifying these risks, and particularly in polar regions where warming is most severe. The biodiversity of high-latitudes, though lower than the tropics, is still being documented, as evidenced by the most recently described species of Alaskan butterfly, *Oeneis tanana* Warren & Nakahara 2016, which was determined to be a species new to science based on examination of museum specimens (Warren *et al.* 2016). I have shown that multiple families of butterfly species are responding morphologically and phenologically to rising temperatures in Alaska, and that the responses are not uniform among species. These results invite further investigation, as arthropods across high-latitudes may exhibit both divergent and dramatic reactions to continued climate warming.

Appendix

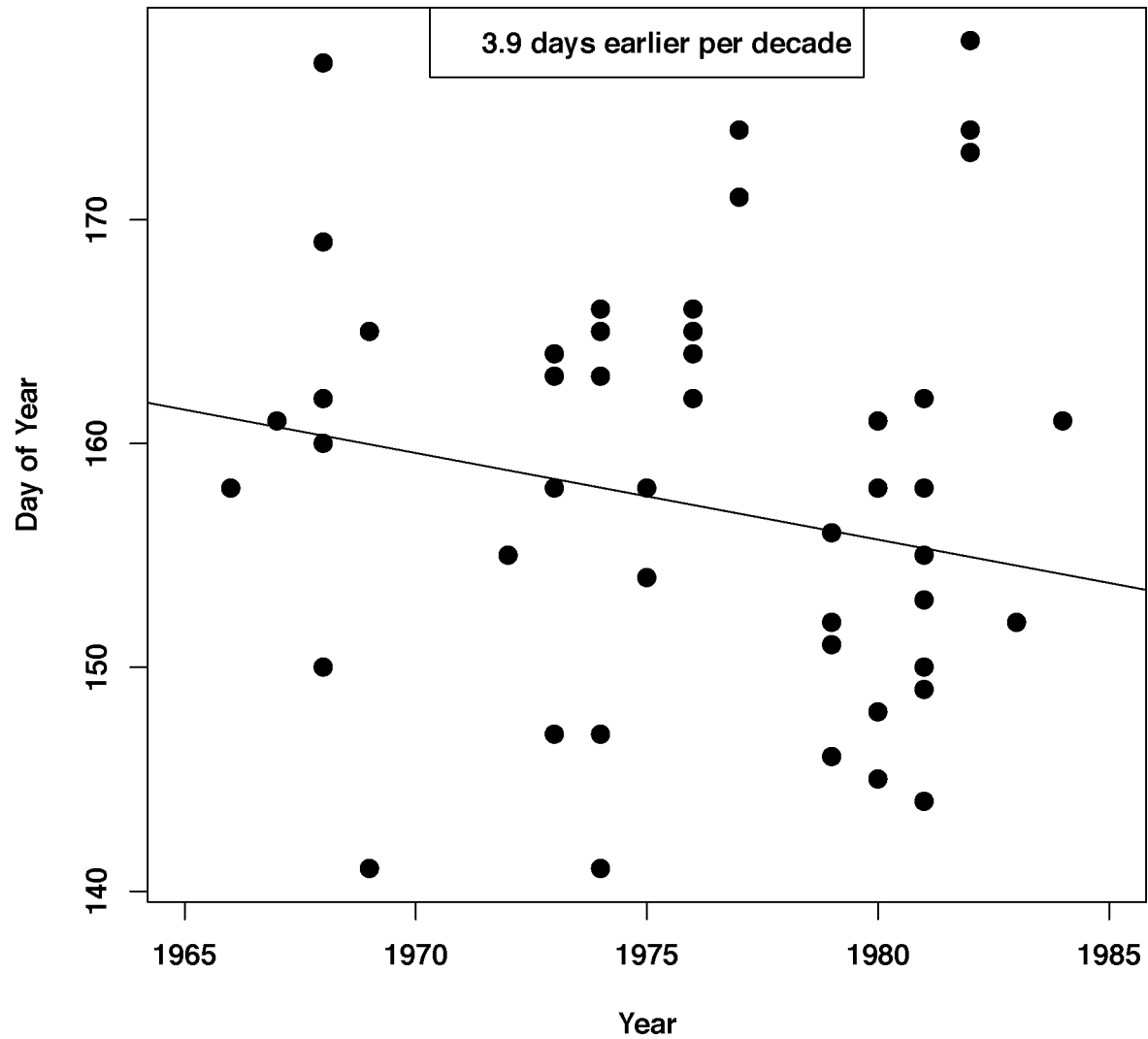


Figure A1. *Erynnis persius* records from 1966-1990 for Fairbanks, Alaska revealed a non-significant advancement of 3.8 ± 1.4 SE days per decade ($R^2 = 0.05146$, $F = 7.052$, $p = 0.008906$).

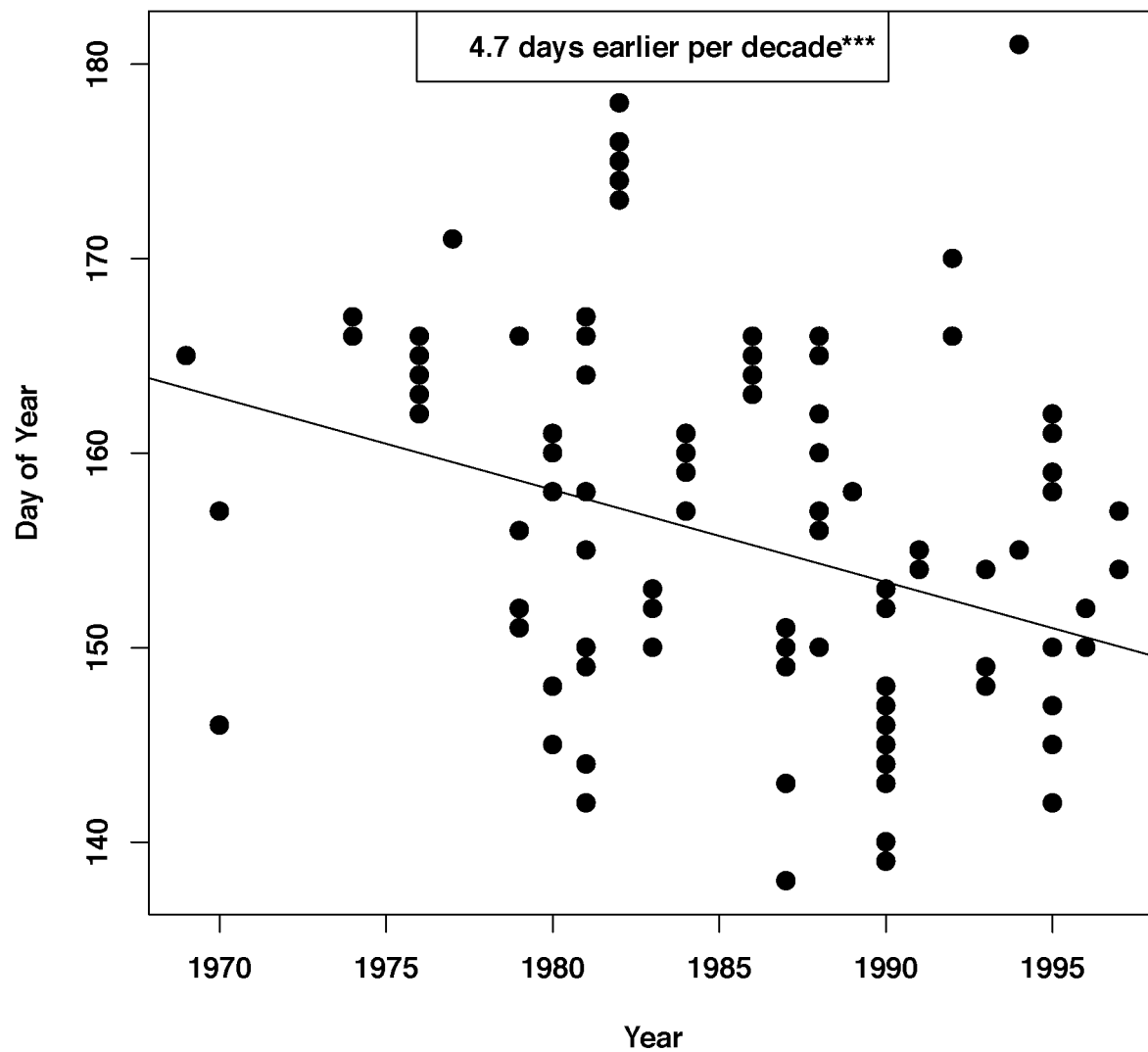


Figure A2. *Colias canadensis* in Fairbanks, Alaska from 1970 – 2005 exhibited a strong advancement of 4.7 ± 0.6 SE days per decade ($R^2 = 0.1002$, $F = 56.82$, $p < 0.001$).

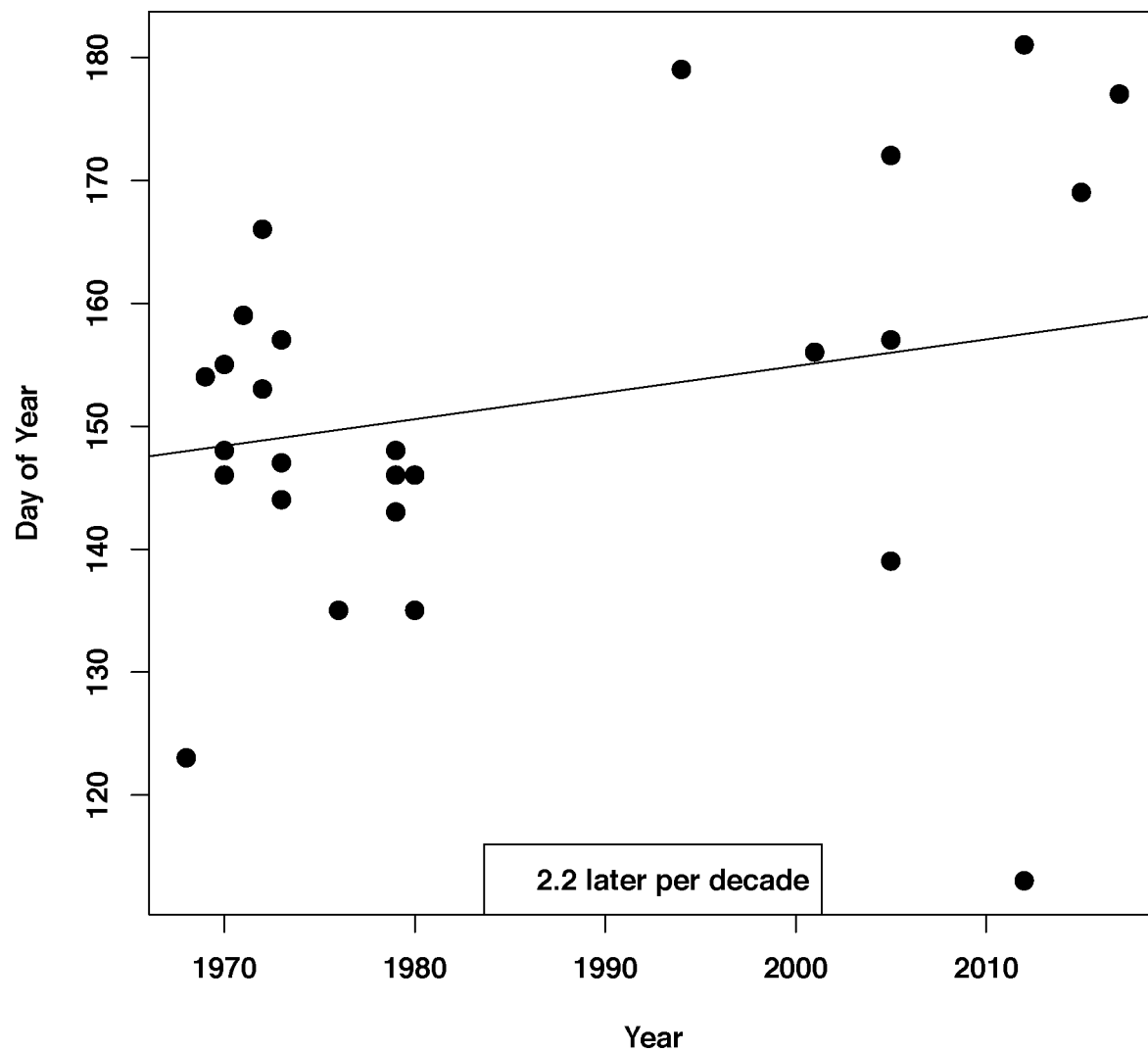


Figure A3. *Pontia occidentalis* in Fairbanks, Alaska from 1966-2016 showed a non-significant delay in flight period, flying 2.2 ± 1.4 SE days later per decade ($R^2 = 0.05944$, $F = 2.275$, $p = 0.1402$) over 51 years.

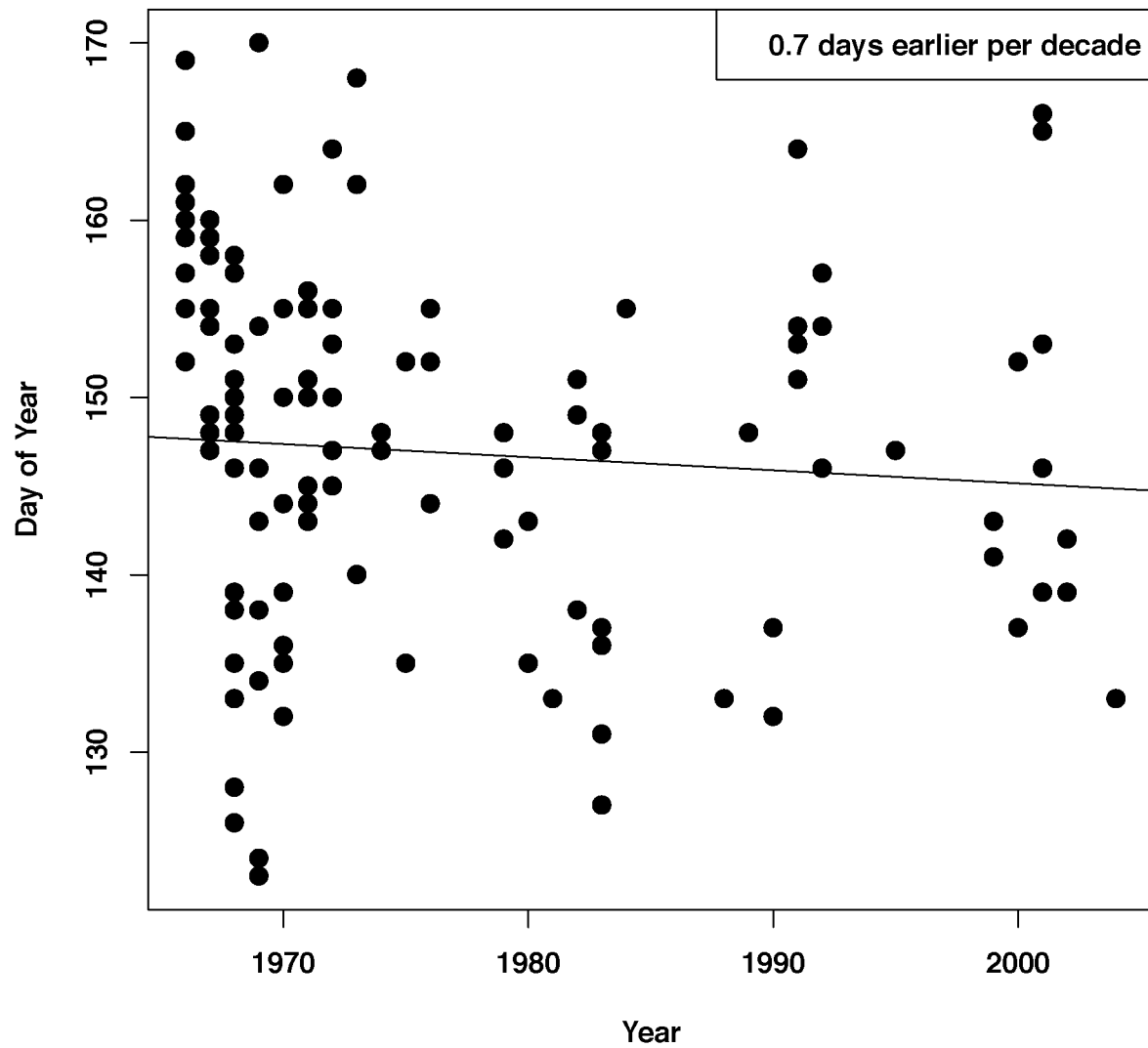


Figure A4. *Culastrina lucia* in Fairbanks, Alaska from 1966-2016 had a non-significant change in flight period over 51 years of 0.7 ± 0.5 SE days later per decade ($R^2 = 0.006735$, $F = 1.553$, $p = 0.214$).

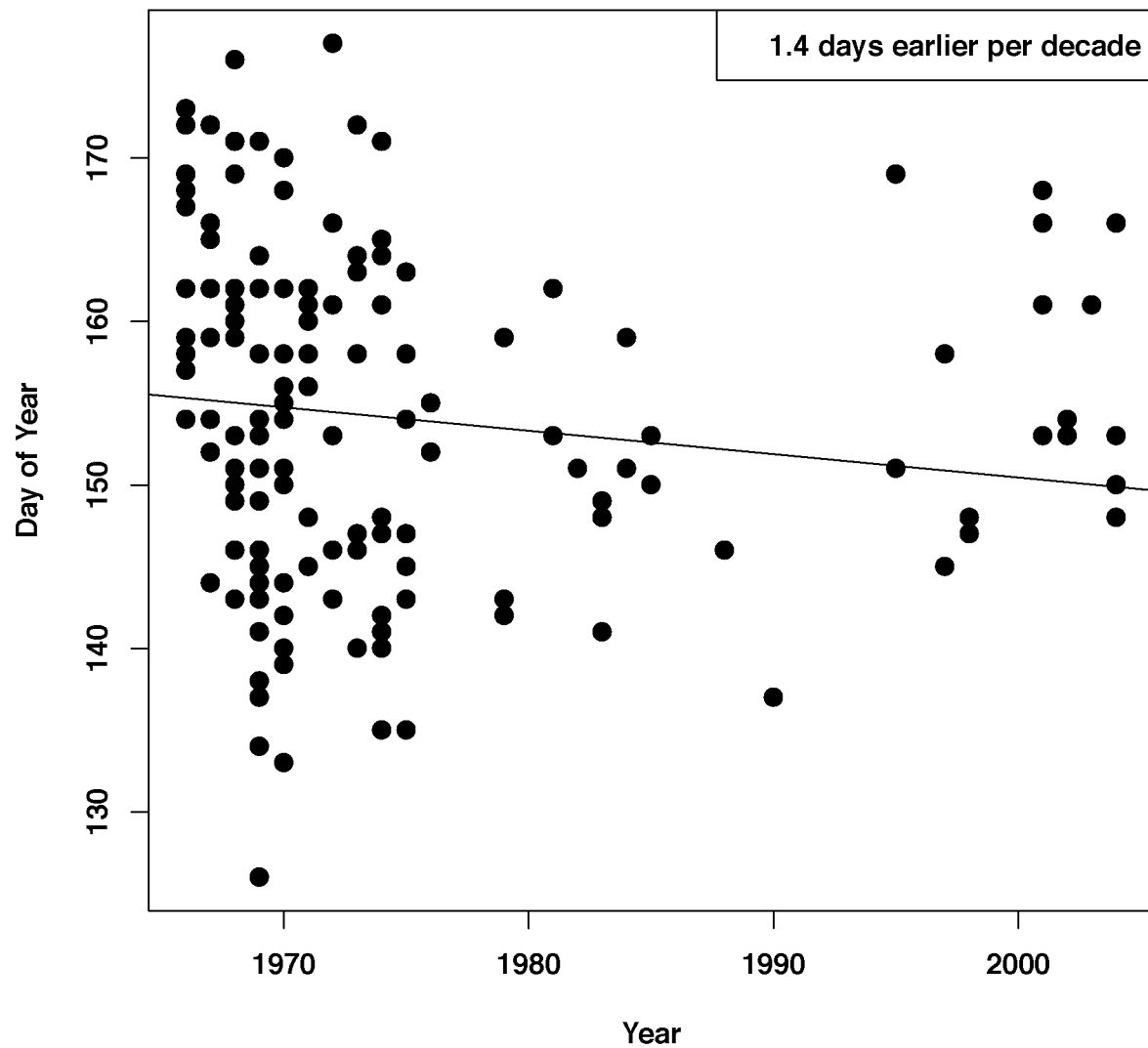


Figure A5. *Glaucopsyche lygdamus* in Fairbanks, Alaska from 1966-2016 advanced 1.4 ± 0.4 SE days per decade ($R^2 = 0.03035$, $F = 10.77$, $p = 0.001138$)

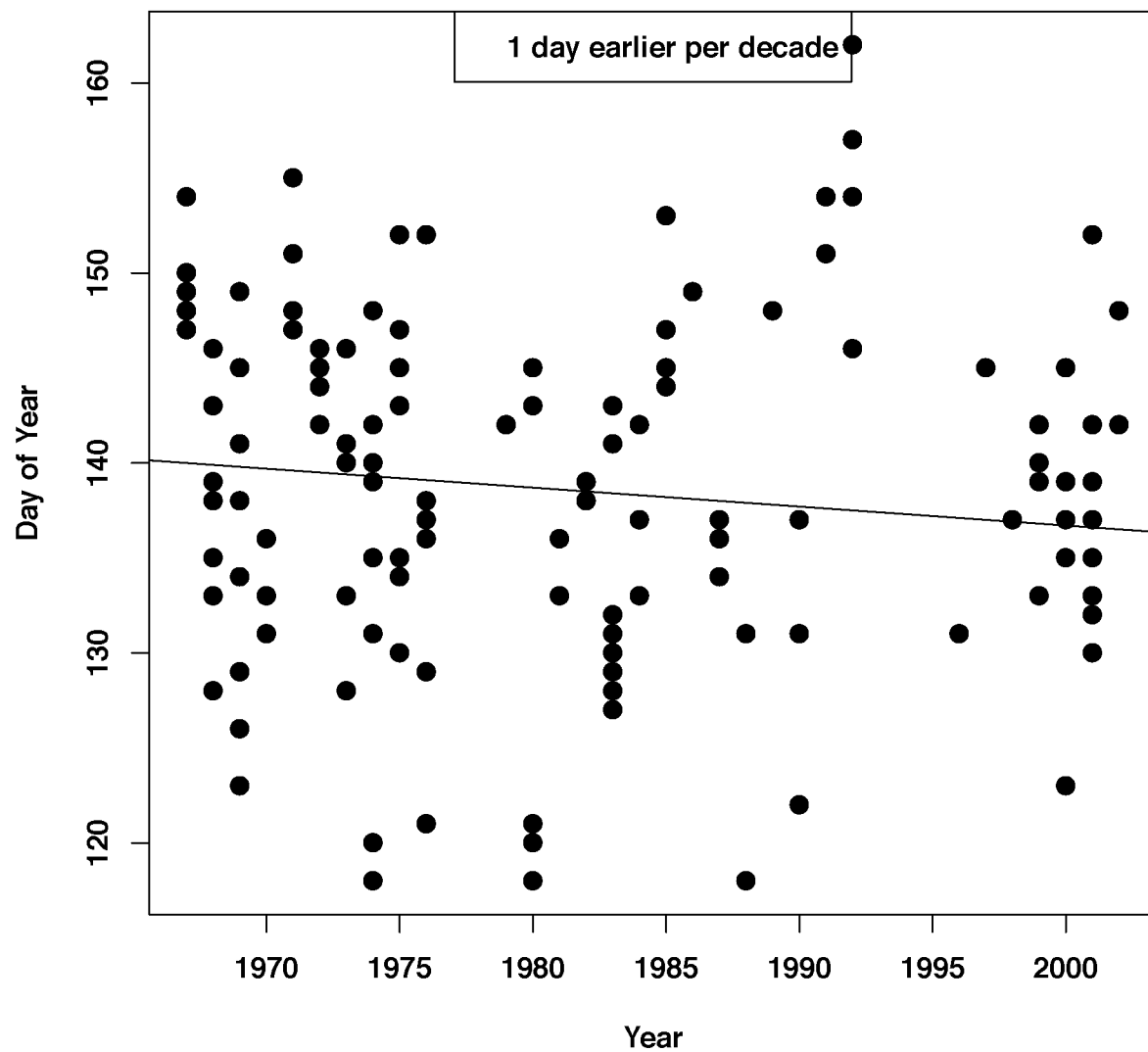


Figure A6. *Callophrys polios* in Fairbanks, Alaska from 1966 – 2005 advanced 1.0 ± 0.4 SE days per decade ($R^2 = 0.01369$, $F = 5.4$, $p = 0.02065$)

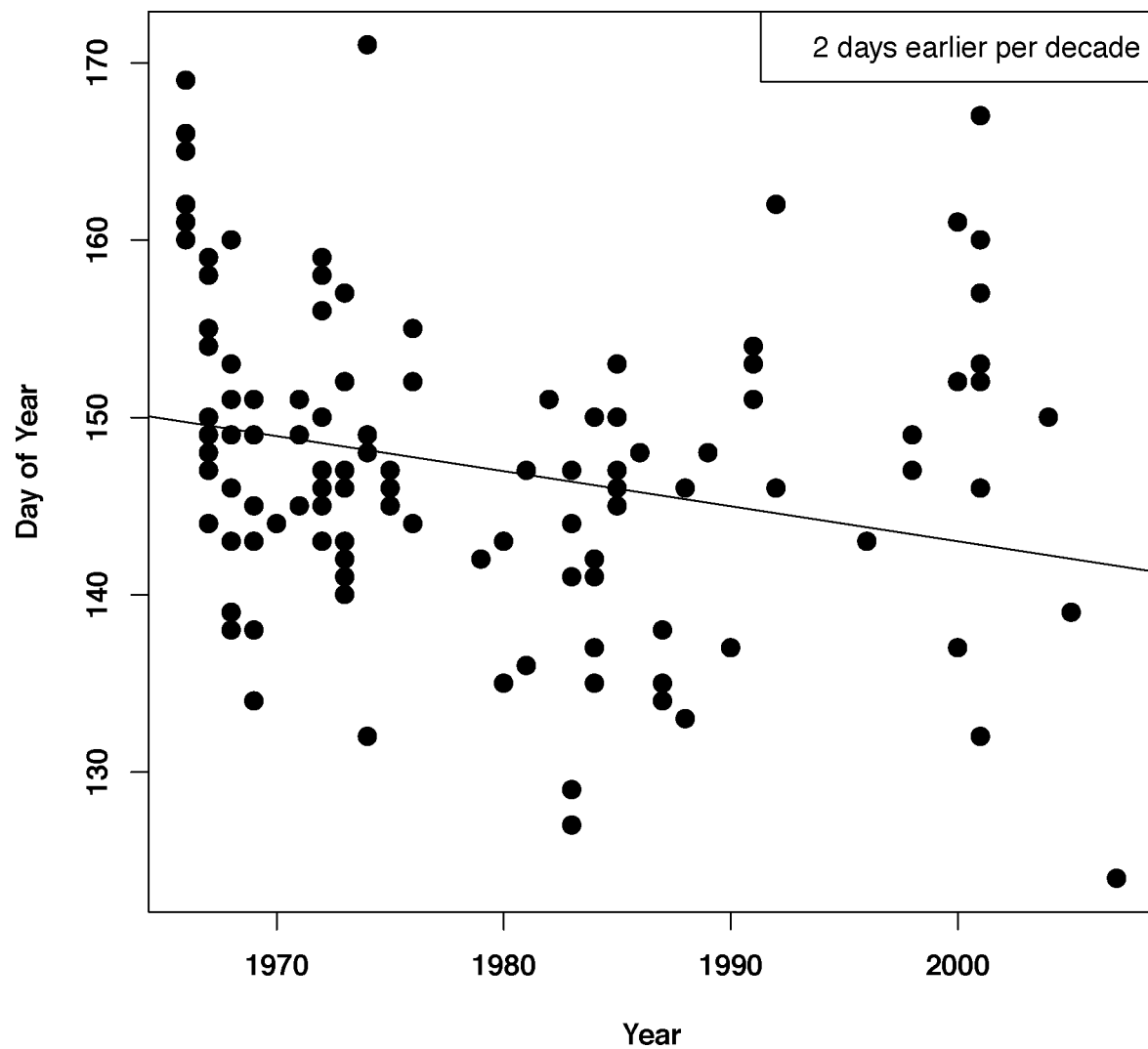


Figure A7. *Callophrys augustinus* in Fairbanks, Alaska from 1966-2005 had an early flight trend of 2.0 ± 0.4 SE days per decade ($R^2 = 0.06262$, $F = 20.17$, $p < 0.001$).

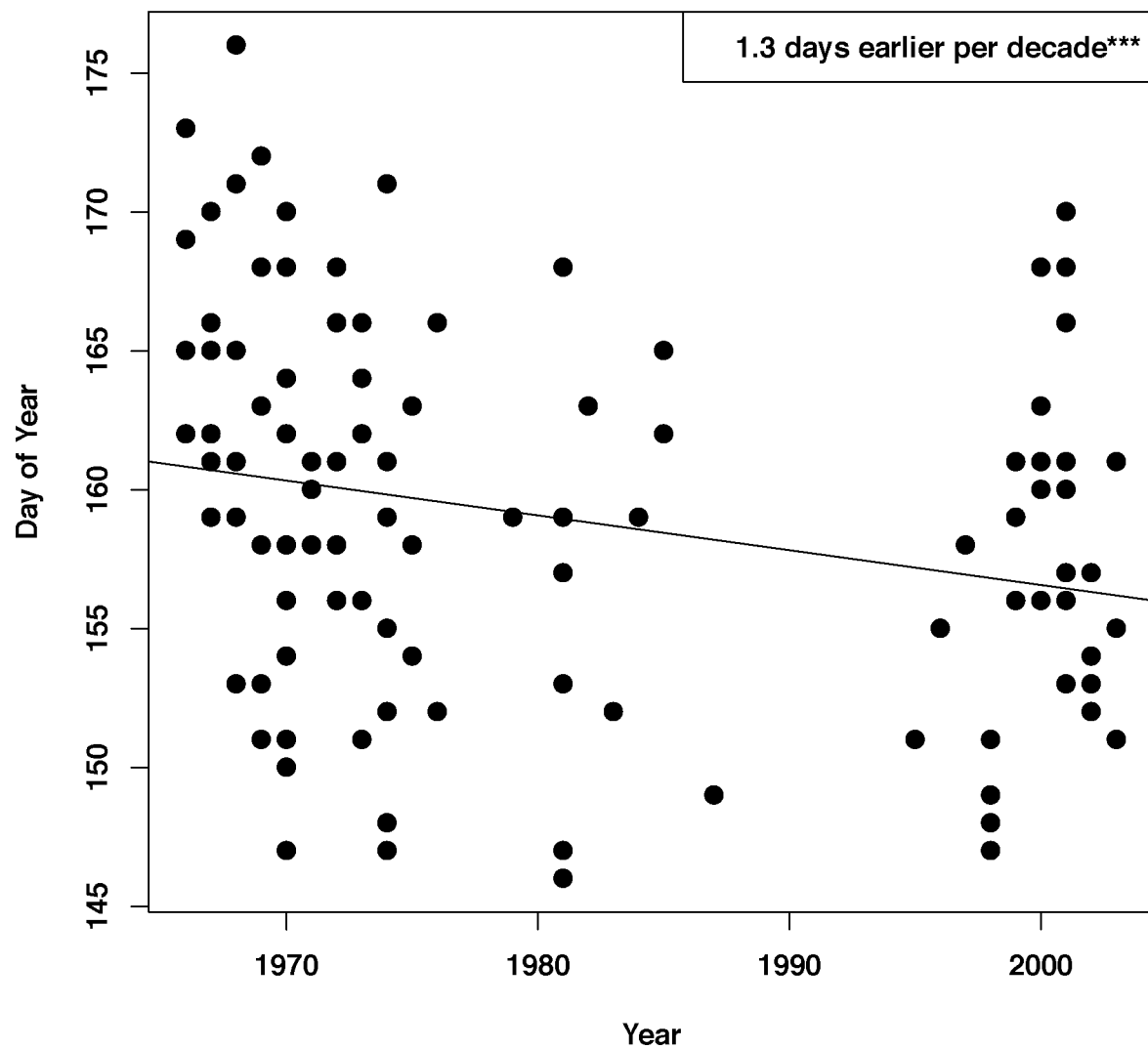


Figure A8. *Boloria frigga* in Fairbanks, Alaska from 1966-2005 advanced 1.3 ± 0.3 SE days per decade ($R^2 = 0.06089$, $F = 18.28$, $p < 0.001$)

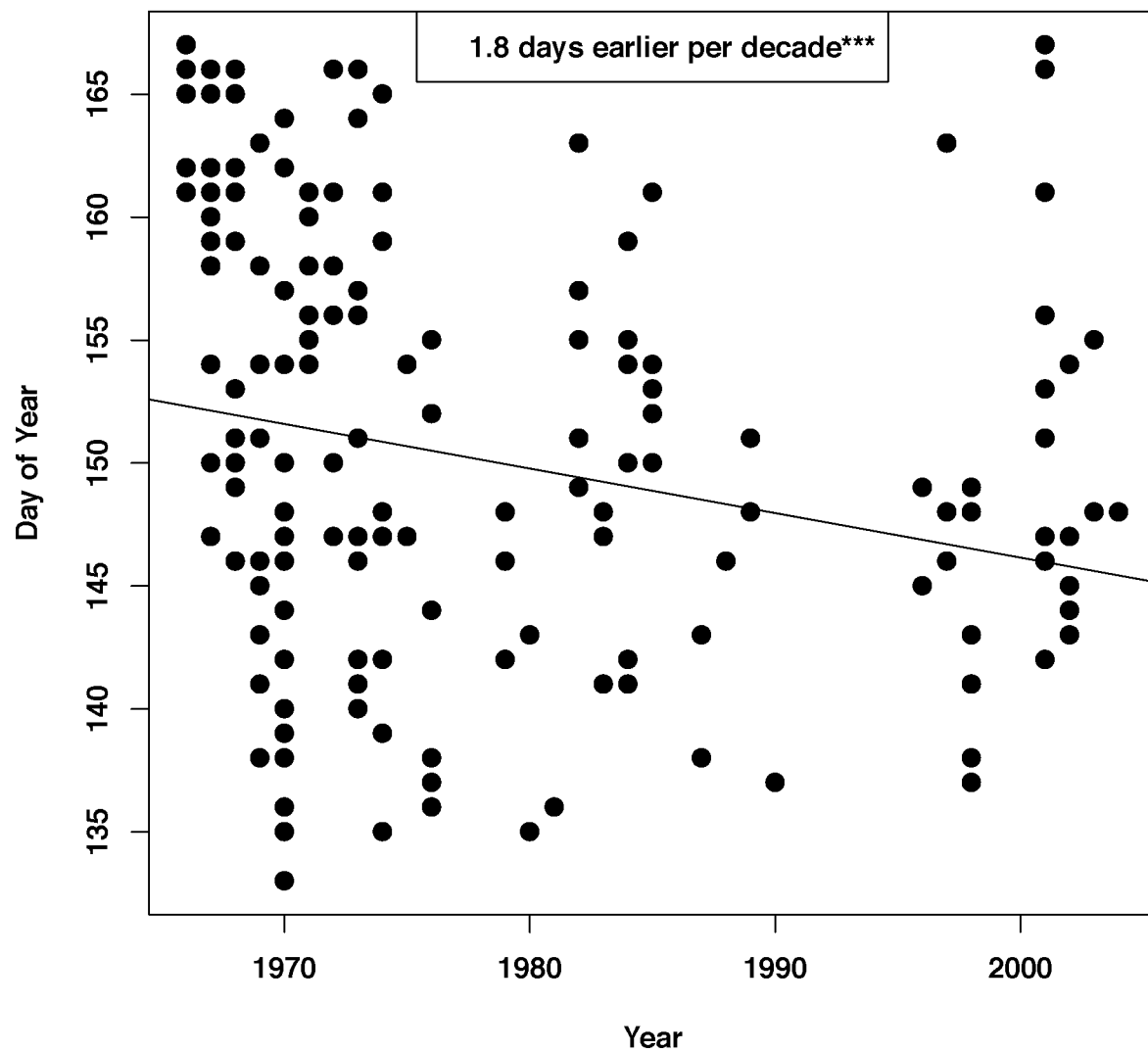


Figure A9. *Boloria freija* in Fairbanks, Alaska from 1966 – 2005 had an advancement of 1.8 ± 0.4 SE days per decade ($R^2 = 0.0485$, $F = 19.83$, $p < 0.001$).

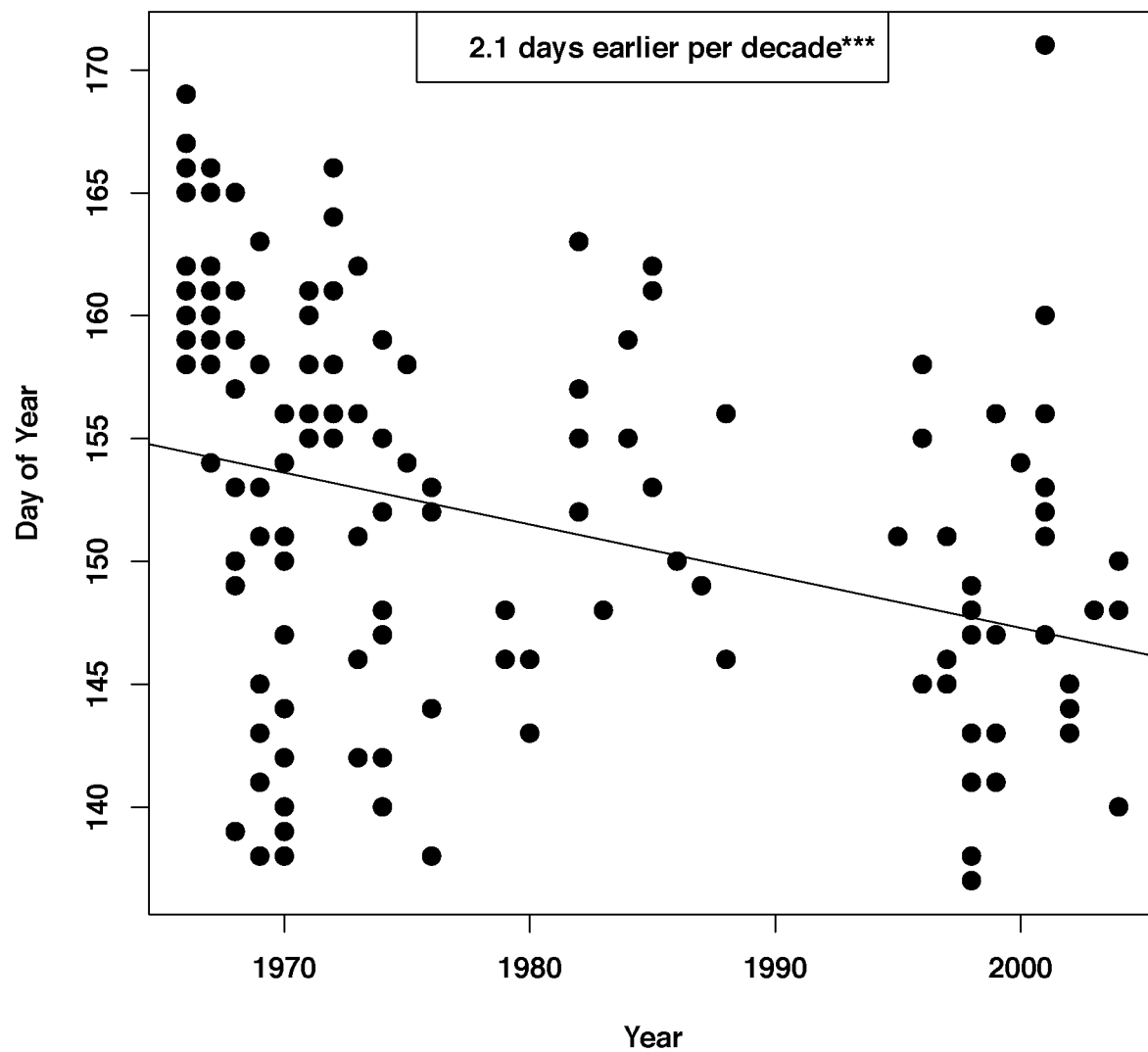


Figure A10. *Erebia discoidalis* in Fairbanks, Alaska from 1966-2016 advanced 2.1 ± 0.3 SE days per decade ($R^2 = 0.1356$, $F = 37.95$, $p < 0.001$).

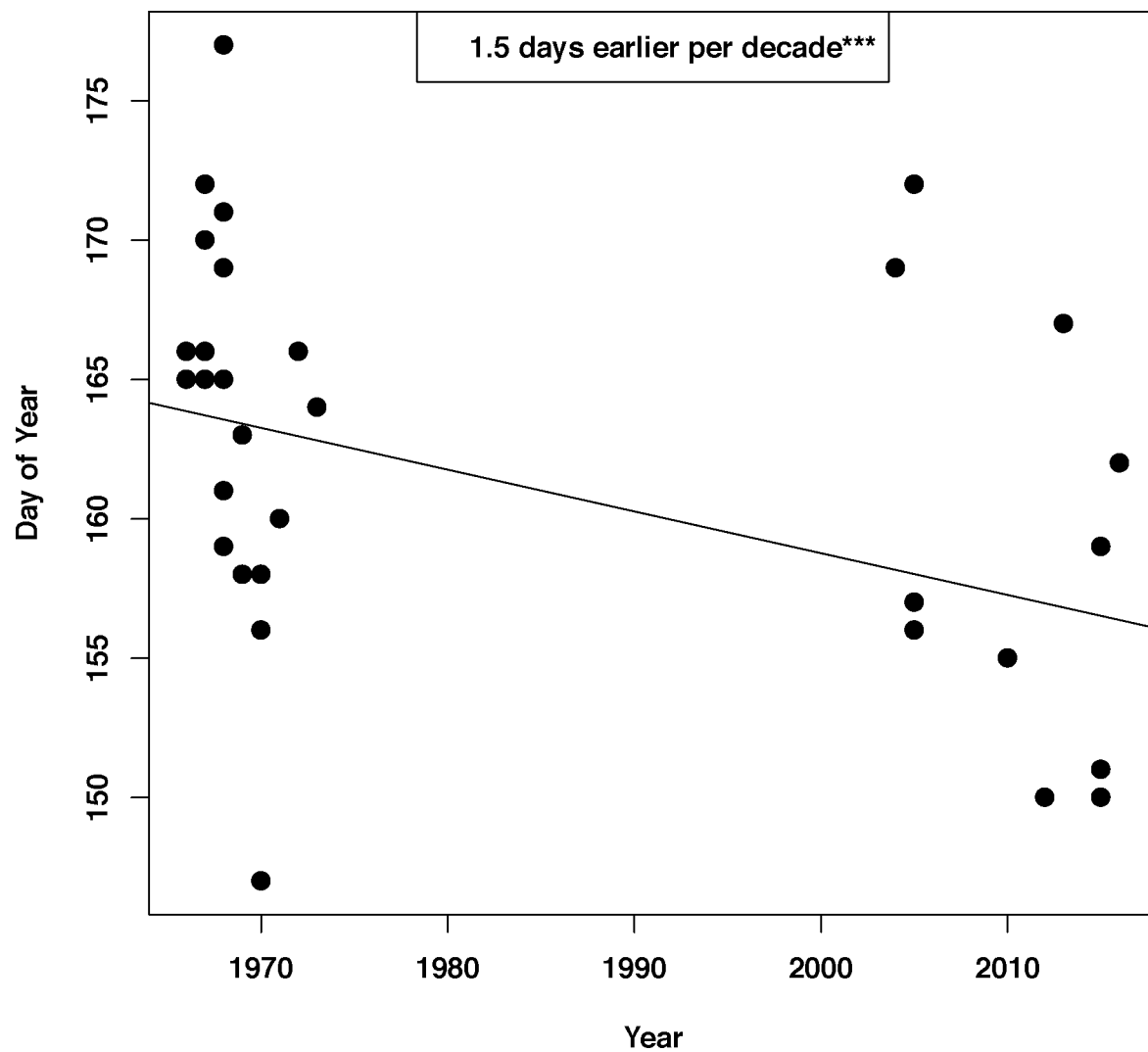


Figure A11. *Erebia epipsodea* in Fairbanks ,Alaska from 1966-2016 advanced 1.5 ± 0.2 SE days per decade ($R^2 = 0.2848$, $F = 40.62$, $p < 0.001$).

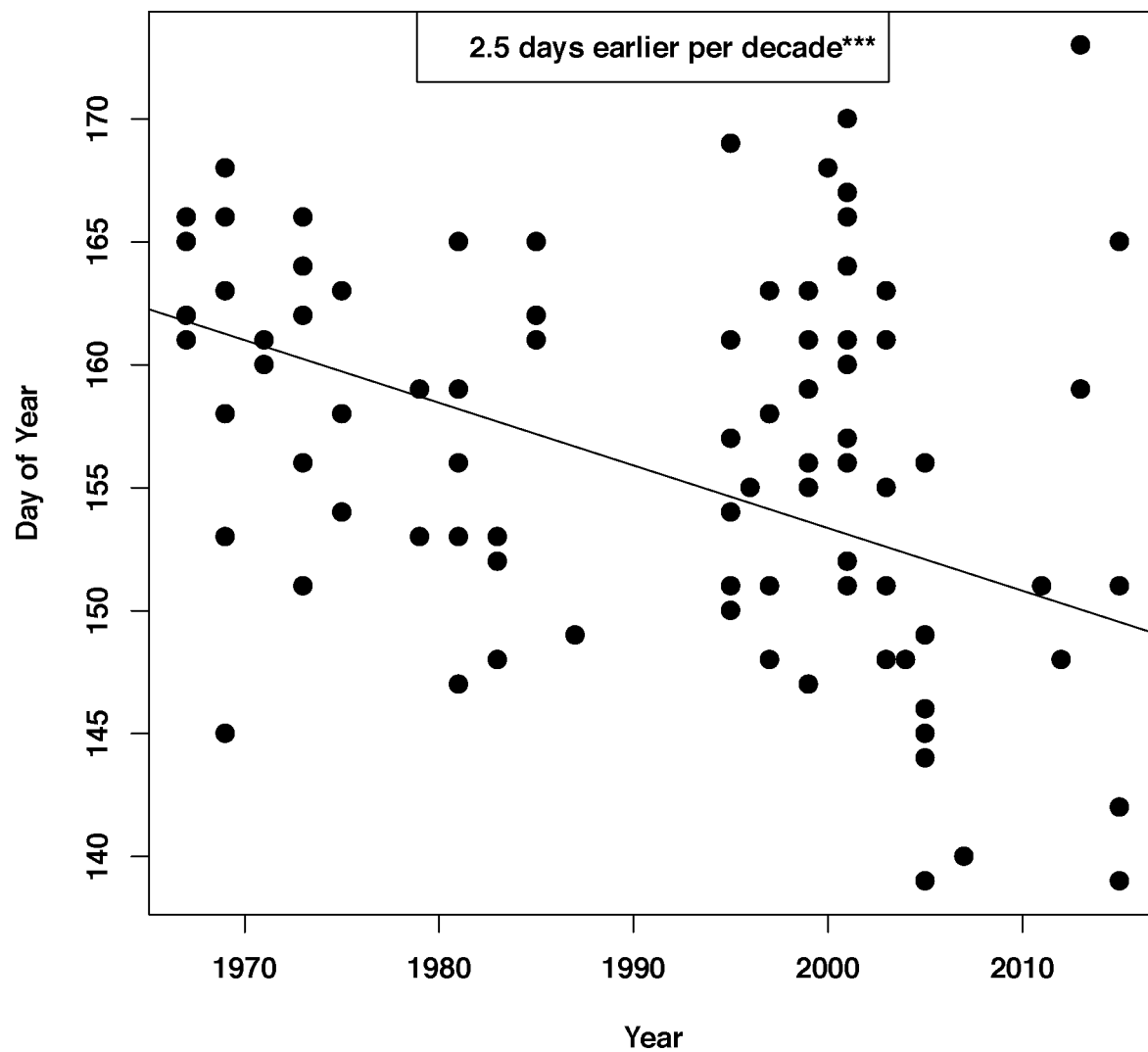


Figure A12. *Erebia mancinus* in Fairbanks, Alaska from 1966-2016 advanced 2.5 ± 0.3 SE days per decade ($R^2 = 0.2218$, $F = 51.31$, $p < 0.001$) from 1966-2017.

Literature Cited

- Altermatt, F. (2010). Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society of London B: Biological Sciences*, 277(1685): 1281-1287.
- Alpatov, W. W. (1929). Biometrical studies on variation and races of the honey bee (*Apis mellifera* L.). *The Quarterly Review of Biology*, 4(1): 1-58.
- Avila-Jiménez, M. L., Coulson, S. J., Solhøy, T., & Sjöblom, A. (2010). Overwintering of terrestrial Arctic arthropods: the fauna of Svalbard now and in the future. *Polar Research*, 29(1), 127-137.
- Beck, P.S., Juday, G.P., Alix, C., Barber, V.A., Winslow, S.E., Sousa, E.E., Heiser, P., Herriges, J.D. and Goetz, S.J., (2011). Changes in forest productivity across Alaska consistent with biome shift. *Ecology letters*, 14(4): 373-379.
- Beckemeyer, R. J., & Hall, J. D. (2007). The entomofauna of the Lower Permian fossil insect beds of Kansas and Oklahoma, USA. *African Invertebrates*, 48(1): 23-39.
- Berger, D., Walters, R., & Gotthard, K. (2008). What limits insect fecundity? Body size-and temperature-dependent egg maturation and oviposition in a butterfly. *Functional Ecology*, 22(3): 523-529.
- Bhatt, U.S., Walker, D.A., Raynolds, M.K., Bieniek, P.A., Epstein, H.E., Comiso, J.C., Pinzon, J.E., Tucker, C.J., Steele, M., Ermold, W. and Zhang, J., (2017). Changing seasonality of panarctic tundra vegetation in relationship to climatic variables. *Environmental Research Letters*, 12(5): 1-18.
- Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers,

- A.P., Potts, S.G., Kleukers, R., Thomas, C.D. and Settele, J., (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313(5785): 351-354.
- Bowden, J. J., Eskildsen, A., Hansen, R. R., Olsen, K., Kurle, C. M., & Høye, T. T. (2015). High-Arctic butterflies become smaller with rising temperatures. *Biology Letters*, 11(10): 1-5
- Braddy, S. J., Poschmann, M., & Tetlie, O. E. (2008). Giant claw reveals the largest ever arthropod. *Biology Letters*, 4(1): 106-109.
- Buckley, L.B., Arakaki, A.J., Cannistra, A.F., Kharouba, H.M. and Kingsolver, J.G., (2017). Insect development, thermal plasticity and fitness implications in changing, seasonal environments. *Integrative and Comparative Biology*, 57(5): 988-998.
- Cane, J. and Tepedino, V., (2001). Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences. *Conservation Ecology*, 5(1): 1-7.
- Carroll, A. L., & Quiring, D. T. (1993). Interactions between size and temperature influence fecundity and longevity of a tortricid moth, *Zeiraphera canadensis*. *Oecologia*, 93(2), 233-241.
- Comiso, J. C., & Hall, D. K. (2014). Climate trends in the Arctic as observed from space. *Wiley Interdisciplinary Reviews: Climate Change*, 5(3): 389-409.
- Culler, L. E., Ayres, M. P., & Virginia, R. A. (2015). In a warmer Arctic, mosquitoes avoid increased mortality from predators by growing faster. *Proceedings of the Royal Society of London*. 282(1815): 1-8.
- Danks, H.V., (1978). Modes of seasonal adaptation in the insects: Winter survival. *Canadian*

- Entomologist*, 110(11): 1167-1205.
- Davidson, J. (1944). On the relationship between temperature and rate of development of insects at constant temperatures. *The Journal of Animal Ecology*, 26-38.
- Dudley, R. (1998). Atmospheric oxygen, giant Paleozoic insects and the evolution of aerial locomotor performance. *Journal of Experimental Biology*, 201(8): 1043-1050.
- Goetz, S. J., Bunn, A. G., Fiske, G. J., & Houghton, R. A. (2005). Satellite-observed photosynthetic trends across boreal North America associated with climate and fire disturbance. *Proceedings of the National Academy of Sciences*, 102(38): 13521-13525.
- Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T. and Goulson, D., (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PloS One*, 12(10): 1-13.
- Harlé, E. (1911). Le vol de grands reptiles et insectes disparus semble indiquer une pression atmosphérique élevée. [translation: The flight of large reptiles and missing insects seems to indicate a high atmospheric pressure] la Sté.
- Harrison, J. F., Kaiser, A., & VandenBrooks, J. M. (2010). Atmospheric oxygen level and the evolution of insect body size. *Proceedings of the Royal Society of London: Biological Sciences*, 277(1690): 1937-1946.
- Høye, T.T., Eskildsen, A., Hansen, R.R., Bowden, J.J., Schmidt, N.M. and Kissling, W.D., (2014). Phenology of high-arctic butterflies and their floral resources: species-specific responses to climate change. *Current Zoology*, 60(2): 243-251.
- Johnson, K.G., Brooks, S.J., Fenberg, P.B., Glover, A.G., James, K.E., Lister, A.M., Michel, E.,

- Spencer, M., Todd, J.A., Valsami-Jones, E. and Young, J.R., (2011). Climate change and biosphere response: unlocking the collections vault. *BioScience*, 61(2): 147-153.
- Jones, R. E., Hart, J. R., & Bull, G. D. (1982). Temperature, size and egg production in the cabbage butterfly, *Pieris rapae*. *Australian Journal of Zoology*, 30(2): 223-231.
- Ju, J., & Masek, J. G. (2016). The vegetation greenness trend in Canada and US Alaska from 1984–2012 Landsat data. *Remote Sensing of Environment*, 176(1): 1-16.
- Juday, G. P., Alix, C., & Grant, T. A. (2015). Spatial coherence and change of opposite white spruce temperature sensitivities on floodplains in Alaska confirms early-stage boreal biome shift. *Forest Ecology and Management*, 350(1): 46-61.
- Kennelly, D., Grigg, J., Tabaru, A. and Sentak, K., (2017). The effects of temperature on *Vanessa cardui* wing size. *Journal of Biological Sciences*, 3(1): 6-8.
- Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., Rasmont, P., Schweiger, O., Colla, S. R., Richardson, L.L., Wagner, D. L., Gall, L. F., Sikes, D. S., Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science* 349(6244): 177-180
- Kharouba, H. M., Paquette, S. R., Kerr, J. T., & Vellend, M. (2014). Predicting the sensitivity of butterfly phenology to temperature over the past century. *Global Change Biology*, 20(2): 504-514.
- Loboda, S., Savage, J., Buddle, C. M., Schmidt, N. M., & Høye, T. T. (2018). Declining diversity and abundance of High Arctic fly assemblages over two decades of rapid climate warming. *Ecography*, 41(2): 265-277.

- MacLean, H. J., Kingsolver, J. G., & Buckley, L. B. (2016). Historical changes in thermoregulatory traits of alpine butterflies reveal complex ecological and evolutionary responses to recent climate change. *Climate Change Responses*, 3(1): 13.
- Masaki, S. (1978). Seasonal and latitudinal adaptations in the life cycles of crickets. *Evolution of Insect Migration and Diapause* (pp. 72-100). Springer, New York, NY.
- Philip, K.W., Ferris, C.D. (2016). Butterflies of Alaska: A Field Guide. 2nd ed. *Alaska Entomological Society*. (p. 110)
- Porcelli, D., Gaston, K. J., Butlin, R. K., & Snook, R. R. (2017). Local adaptation of reproductive performance during thermal stress. *Journal of Evolutionary Biology*, 30(2): 422-429.
- Posledovich, D., Toftegaard, T., Navarro-Cano, J. A., Wiklund, C., Ehrlén, J., & Gotthard, K. (2014). Latitudinal variation in thermal reaction norms of post-winter pupal development in two butterflies differing in phenological specialization. *Biological Journal of the Linnean Society*, 113(4): 981-991.
- Post, E., Steinman, B. A., & Mann, M. E. (2018). Acceleration of phenological advance and warming with latitude over the past century. *Scientific Reports*, 8(1): 3927.
- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6): 345-353.
- Przybylak, R. (2007). Recent air-temperature changes in the Arctic. *Annals of Glaciology*, 46(1): 316-324.
- Richter-Menge, J., Overland, J. E., & Mathis, J. T. (2016). *Arctic Report Card*, 2016.
- Rigor, I. G., Colony, R. L., & Martin, S. (2000). Variations in surface air temperature

- observations in the Arctic, 1979–97. *Journal of Climate*, 13(5): 896-914.
- Roy, D.B. and Sparks, T.H., (2000). Phenology of British butterflies and climate change. *Global Change Biology*, 6(4): 407-416.
- Seltmann, K. C., Cobb, N. S., Gall, L. F., Bartlett, C. R., Basham, M. A., Betancourt, I., Bills, C., Brandt, B., Brown, R. L., Bundy, C., Caterino, M. S., Chapman, C., Cognato, A., Colby, J., Cook, S. P., Daly, K. M., Dyer, L. A., Franz, N. M., Gelhaus, J. K., Grinter, C. C., Harp, C. E., Hawkins, R. L., Heydon, S. L., Hill, G. M., Huber, S., Johnson, N., Kawahara, A. Y., Kimsey, L. S., Kondratieff, B. C., Krell, F. T., Leblanc, L., Lee, S., Marshall, C. J., McCabe, L. M., McHugh, J. V., Menard, K. L., Opler, P. A., Palffy-Muhoray, N., Pardikes, N., Peterson, M. A., Pierce, N. E., Poremski, A., Sikes, D. S., Weintraub, J. D., Wikle, D., Zaspel, J. M., and Zolnerowich, G. (2017). LepNet: The Lepidoptera of North America Network. *Zootaxa*, 4247(1): 73-77.
- Sikes, D.S., Bowser, M., Daly, K., Høye, T., Meierotto, S., Mullen, L., Slowik, J. & Stockbridge, J. (2017). The value of museums in the production, sharing, and use of entomological data to document hyperdiversity of the changing North. *Arctic Science* 3: 498-514.
- Singer, M.C. and Parmesan, C., (2010). Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy? *Philosophical Transactions of the Royal Society of London: Biological Sciences*, 365(1555): 3161-3176.
- Stefanescu, C., Penuelas, J. and Filella, I., (2003). Effects of climatic change on the phenology of butterflies in the northwest Mediterranean Basin. *Global Change Biology*, 9(10): 1494-1506.
- Sturm, M., Racine, C., & Tape, K. (2001). Climate change: increasing shrub abundance in the Arctic. *Nature*, 411(6837): 546.

- Sturm, M., Schimel, J., Michaelson, G., Welker, J.M., Oberbauer, S.F., Liston, G.E., Fahnestock, J. & Romanovsky, V.E., (2005). Winter biological processes could help convert arctic tundra to shrubland. *AIBS Bulletin*, 55(1): 17-26.
- Stone, R. S., Dutton, E. G., Harris, J. M., & Longenecker, D. (2002). Earlier spring snowmelt in northern Alaska as an indicator of climate change. *Journal of Geophysical Research: Atmospheres*, 107(10): 1-10.
- Suarez, A. V., & Tsutsui, N. D. (2004). The value of museum collections for research and society. *AIBS Bulletin*, 54(1): 66-74.
- Tauber, M. J., Tauber, C. A., Masaki, S. (1986). Seasonal adaptations of insects. *Oxford University Press*. (p. 414)
- Thackeray, S.J., Sparks, T.H., Frederiksen, M., Burthe, S., Bacon, P.J., Bell, J.R., Botham, M.S., Brereton, T.M., Bright, P.W., Carvalho, L. and Clutton-Brock, T. (2010). Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology*, 16(12): 3304-3313.
- Temperli, C., Veblen, T. T., Hart, S. J., Kulakowski, D., & Tepley, A. J. (2015). Interactions among spruce beetle disturbance, climate change and forest dynamics captured by a forest landscape model. *Ecosphere*, 6(11): 1-20.
- Tseng, M., Kaur, K.M., Soleimani Pari, S., Sarai, K., Chan, D., Yao, C.H., Porto, P., Toor, A., Toor, H.S. and Fograscher, K., (2018). Decreases in beetle body size linked to climate change and warming temperatures. *Journal of Animal Ecology*, 87(3): 647-659.
- Van Dyck, H., Bonte, D., Puls, R., Gotthard, K., & Maes, D. (2015). The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? *Oikos*, 124(1): 54-61.

- Vermeij, G. J. (2016). Gigantism and its implications for the history of life. *PLoS One*, 11(1): 1-8
- Warren, A. D., Nakahara, S., Lukhtanov, V., Daly, K., Ferris, C., Grishin, N., Cesanek, M., Pelham, J. (2016). A new species of *Oeneis* from Alaska, United States, with notes on the *Oeneis chryxus* complex (Lepidoptera: Nymphalidae: Satyrinae). *The Journal of Research on the Lepidoptera*, 49(1): 1–20.
- Westwood, A.R. and Blair, D., (2010). Effect of regional climate warming on the phenology of butterflies in boreal forests in Manitoba, Canada. *Environmental entomology*, 39(4): 1122-1133.
- Williams, E.H., Stichter, S.B., Hitchcock, C., A. Polgar, C. and Primack, R.B. (2014). Phenological advancement of Lycaenid butterflies in Massachusetts. *The Journal of the Lepidopterists' Society*, 68(3): 167-174.